VOL.
31
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VOL
31
195

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VOL.
31
1950

INNEHÅLL

OLE VENGE: Studies of the Maternal Influence on the Birth Weight in Rabbits . . 1

LARS SILÉN: On the Nervous System of Glossobalanus marginatus Meek (Enteropneusta) 149

ALF G. JOHNELS: On the Dermal Connective Tissue of the Head of Petromyzon . . . 177

PAUL WALDER: Über das Wachstum der Kopforgane und die Entstehung von Mikrophthalmie bei isolierten Kopfstücken und zirkulationslosen Keimen von Triton alpestris 187

NILS HOLMGREN: On the Pronephros and the Blood in Myxine Glutinosa 233

LARS SILÉN: On the Mobility of Entire Zoids in Bryozoa 349

VOL.
31
1950

STUDIES OF THE MATERNAL INFLUENCE ON THE BIRTH WEIGHT IN RABBITS

BY

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OL.
31
950

PREFACE.

In presenting this paper I must first and foremost emphasize how deeply obliged I am to Prof. IVAR JOHANSSON, Director of the Institute of Animal Breeding, Royal Agricultural College of Sweden. During a visit to the Institute in October 1945 Prof. Johansson suggested to me the problem which has formed the subject of these studies and gave me the opportunity of commencing the experimental work in the Spring of 1946. Since then four years have gone by, during which time I have had admirable facilities for carrying out the work. I should like to thank Prof. Johansson for the kindness with which he always has placed assistance at my disposal whenever it became necessary, and I am indepted to him for allowing me to use the stock of rabbits belonging to the Institute. I further wish to express my gratitude to him for our frequent discussions and his continued interest throughout the course of the work.

Throughout the experiments I have had an invaluable assistant in Miss RAGNHILD HALSE, who has helped me with the operations with unique interest and efficiency. In the statistical treatment of the data Mr. PETER GOLDSTEIN has done much careful routine work. The daily care of the experimental animals has been painstakingly attended to by Messrs. AXEL JANSSON and OLLE JANSSON. The chemical analyses have been done by Mr. ALLAN FRÖLICH, B. Sc. Some of the photographs were taken by the veterinarian Mr. IVAR DYRENDAHL, while the drawings have been made by Mr. ARVIDS UDRIS, B. Sc. I most sincerely thank all these persons for the way in which they have facilitated my work.

I am also indebted to Mr. HALFDAN MADSEN, M. A., who undertook to translate the manuscript into English. Subsequently the manuscript has been

carefully perused by Dr. GRAEME WILSON, of Australia. The Danish manuscript has been read by Mr. JOEL NELSON, B. Sc., who has pointed out several obscurities. Dr. LARS FRYKHOLM and Miss ASTRID GUILLETMOT, B. A., of the Library of the Royal Agricultural College have rendered a valuable assistance in obtaining literature. To these persons I am most grateful.

Such large scale investigations as these reported here are only possible with considerable financial support. Valuable help has been received from the *Agricultural Research Council*, Stockholm, the *Academy of Technical Science*, Copenhagen and the *Royal Veterinary and Agricultural College*, Copenhagen. In addition, Professor JOHANSSON has personally made a substantial contribution to this end.

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VOL.
31
1950

CONTENTS.

Preface 1
Introduction 5
Review of Literature Concerning the Inheritance of Size in Mammals 6
Rabbits 7
Other mammals 15
Material and Methods of the Present Investigation 19
Material 20
Animals 20
Feeding 23
Health 24
Methods 24
General conditions 24
Technique of transplantation of fertilized ova 25
Pregnancy Results of the Transplantations 29
Observations on the Physiology of Reproduction 32
a. Ovulation 32
b. Division of the ova 35
c. Implantation and pregnancy 38
A_{II}. Large race borne by Polish does 39
B_{II}. Polish borne by Large race does 40
C_{II}. F_1-crossbreds from Polish ♂ × Large race ♀, borne by Polish does . . 42
D_{II}. F_1-crossbreds from Large race ♂ × Polish ♀, borne by Large race does . 43
E. Pure breeds borne by crossbred does 43
d. Sex ratio 43
Experimental Results 45
Influence of Different Factors on the Birth Weight 45
a. Length of gestation period 46
b. Seasonal influence 50
c. Age and litter number 51
d. Size of the mother 52
α. Effect on the total production of young 52
β. Effect on the size of the individual young 53
A. Large race 54
B. Polish 57
C. SL-crossbreds 58
D. LS-crossbreds 59
e. Sex 59
f. Litter size 61
g. Liveborn and stillborn 68
h. Discussion of the factors influencing birth weight 71
1. General survey 72
2. Special survey 73
Analyses of Variation in the Birth Weight 77
A. Large race 77
B. Small race 82
C. F_1-crossbreds from Polish ♂ × Large race ♀ 85
D. F_1-crossbreds from Large race ♂ × Polish ♀ 88
E. Pure breeds borne by crossbreds 92
F. Crossbreds borne by crossbreds 96
Comparison of the Results from the Different Groups 97
I. Differences in uterine environment 102
$A_I + A_{II}$. Normal and transplanted Large race 102
$B_I + B_{II}$. Normal and transplanted Small race 106
$C_I + C_{II}$. Normal and transplanted SL-crossbreds 110
$D_I + D_{II}$. Normal and transplanted LS-crossbreds 112
II. Cytoplasmic differences 116
$C_I + D_{II}$. Normal SL-crossbreds and transplanted LS-crossbreds 116

$C_{II} + D_I$. Transplanted SL-crossbreds and normal LS-crossbreds 117
F. Reciprocal crossbreds borne by crossbreds 119
III. Genetic differences 119
$A_I + B_{II}$. Normal Large race and transplanted Small race 119
$A_{II} + B_I$. Transplanted Large race and normal Small race 120
$A_I + C_I$. Normal Large race and normal SL-crossbreds 121
$A_{II} + C_{II}$. Transplanted Large race and transplanted SL-crossbreds 122
$A_I + D_{II}$. Normal Large race and transplanted LS-crossbreds 122
$A_{II} + D_I$. Transplanted Large race and normal LS-crossbreds 123
$B_I + C_{II}$. Normal Small race and transplanted SL-crossbreds 124
$B_{II} + C_I$. Transplanted Small race and normal SL-crossbreds 125
$B_I + D_I$. Normal Small race and Normal LS-crossbreds 126
$B_{II} + D_{II}$. Transplanted Small race and transplanted LS-crossbreds 126
E. Large and Small race borne by crossbreds simultaneously 127
Discussion 129
Appendix 139
Chemical and Histological Examinations of Stillborn Fetuses 139
Summary 141
Literature 144

VOL.
31
1950

INTRODUCTION.

Experimental investigations into the inheritance of body size in mammals have largely been carried out in the form of crossbreeding experiments on smaller rodents, partly because their generation interval is comparatively short, and partly because in multiparous animals it is easier to eliminate some of the non-genetic causes of variation. In regard to larger domestic animals statistical investigations have made important contributions towards ascertaining the proportion of the total size variation in a population which is hereditarily determined. The crossbreeding experiments all lead to the same general conclusion, viz. that the first generation crossbreds are intermediate in size between the two parental breeds. Further it has been shown that, as a general rule, the F_1-generation is less variable than the F_2, whereas the total variation of the two parental breeds is usually greater than that of the F_2-generation. When the F_1-animals are backcrossed to either of the parental breeds the offspring is intermediate between the F_1- and P-generations although in this respect some irregularities frequently appear. On the basis of these results it is generally agreed that body size is determined by a large number of genes with an additive effect. However, like other quantitative characters, size is to a large extent subject also to environmental influences.

The fact that the offspring of reciprocal crosses between two breeds of different sizes is nearer to the size of the dam than to that of the sire, has not until recently aroused any particular attention, although it was observed in some of the early experimental enquiries into the problem of size inheritance.

For the purpose of elucidating further the problem of size inheritance in mammals, and in order to make an attempt to throw light on the nature of the maternal influence in the F_1-generation, a series of investigations with crosses between a large and a small sized breed of rabbits, viz. Flemish Giant and Polish, was carried out during the years 1938—1945 by Professor IVAR JOHANSSON at the Intitute of Animal Breeding. The main results of these experiments with regard to the size differences between the reciprocal crosses are shown by the growth curves in Fig. 1 together with the corresponding curves for the parental breeds. The difference between the reciprocal crosses is maintained throughout the growth period, and in the full grown animals it amounts to about 500 gms.

The observed differences between the reciprocal crosses, noticable already at birth and generally maintained throughout the post-natal development, may be due to purely maternal influence or they may be attributable to cytoplasmic inheritance in the proper sense of the word.

By means of ordinary crosses it is impossible to distinguish between cyto-

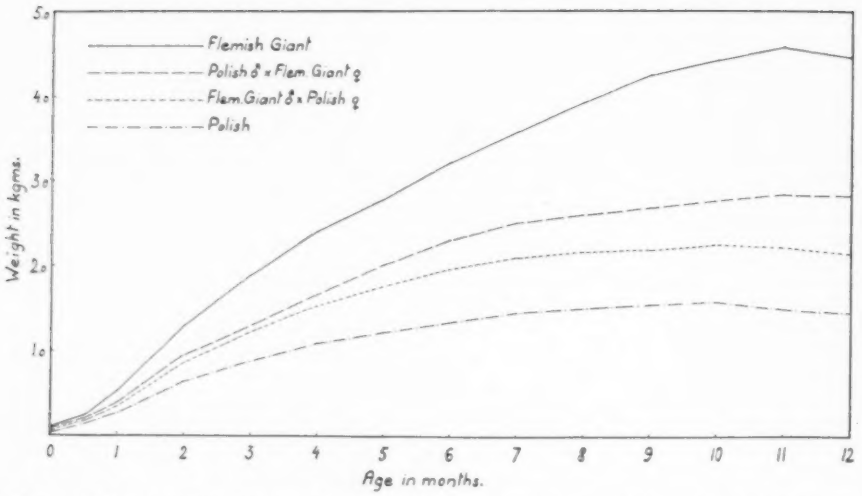

Fig. 1. Growth curves for reciprocal crossbreds between Flemish Giant and Polish and the corresponding curves for the pure breeds.

VOL. 31 1950

plasmic inheritance and modifying maternal influences during the intra-uterine development of the fetuses, nor is there any possibility of ascertaining how much the birth weight may thus be modified. It was therefore decided to study the effect of reciprocal transplantations of fertilized ova from pure breeds and crosses. By this method it should be possible to separate cytoplasmic inheritance, if any, from modifications due to the intra-uterine environment. The present writer has been in charge of this phase of the work since its beginning in February 1946.

This paper deals only with the maternal influence on the weight of the fetuses at birth, whereas the post-natal influence will be treated in a subsequent publication, when a larger number of experimental animals are available in some of the critical groups.

Before the experimental results are considered in detail, it is intended to discuss certain observations pertaining to the physiology of reproduction which were made during the course of the work. However, it must be emphasized that no direct investigations bearing on this subject have been carried out, as this would be beyond the scope of the problem under concideration.

REVIEW OF LITERATURE CONCERNING THE INHERITANCE OF SIZE IN MAMMALS.

During the last thirty or forty years several workers have made extensive investigations into the problem of size inheritance in both uniparous and multiparous mammals. In the following a survey will be given of the most important

results of these studies. In view of the extensive material available it will be necessary, except in the case of rabbits, to confine this review to the inheritance of birth weight.

RABBITS.

In an investigation into the inheritance of certain morphological characteristics in rabbits made by CASTLE (1909), some information is given on the inheritance of size. CASTLE crossed a short-eared breed of rabbits weighing between 2.25 and 2.75 kilos with a long-eared race whose weight ranged from 3.50 to 4.50 kilos, and ascertained that the weight of the offspring was, in general, the mean of those of the two parental breeds. CASTLE drew the conclusion that the adult weight showed blending inheritance, as in his opinion it was not possible to prove either dominance or segregation. From this investigation it appears that the body weight is a comparatively unsatisfactory size criterion, for which reason, instead of exclusively using the difference between the weights of the individual animals as a means of expressing a hereditarily determined difference, MACDOWELL (1914) relies on direct measurements of skeletal parts of adult rabbits, using 17 different measurements in all. A detailed account of MACDOWELL's results will not be given, but it will be sufficient to mention that one of his conclusions was that: "The second generation of a size cross shows greater diversity than does the first generation or the parental lines. Size characters that are inherited in a Mendelian fashion are included in the above statement."

In order to find out to what extent the variation in the different skeletal parts examined by MACDOWELL is genetically determined, CASTLE (1914) calculated the coefficients of correlation between the most important of them. On the basis of the high coefficients found, CASTLE concluded that size is to a very large extent determined by "general factors", i.e. factors influencing all parts of the skeleton simultaneously, but in addition, there is a possibility that various "special factors" operate. These "special factors", however, are only supposed to be of minor importance. He writes: "... What makes the difference between a big rabbit and a small one is clearly a quantitative difference in these general factors, or it is something which modifies the action of these general factors, which amounts to the same thing. That which determines the adult size of a rabbit determines ... both the rate at which it grows and the time when growth ceases."

Similarly, in a cross-experiment with Flemish Giant and Polish, PUNNETT and BAILEY (1918) drew the conclusion that body size (= weight) was intermediately hereditary. Later, CASTLE (1922) used Polish, Himalaya, and Flemish Giant rabbits for the purpose of studying, among other things, the inheritance of size and found that on the average the F_1 ranged somewhat above the exact intermediate weight. This result is partly explained by the fact that

the weight of the Flemish Giant was rather low, which may indicate that the animals used were not fullgrown, or that they were kept on a low plane of nutrition. Examination of the figures given by CASTLE in Table 3 shows that the F_1-generations are of purely intermediate size. At the same time, this table shows that in spite of a somewhat smaller mean weight than the F_1-generation, the F_2 shows a standard deviation that is greater than that of the first cross-bred generation. PEASE (1928) considers the somewhat smaller mean weight of the F_2-generation to be due to heterosis disappearing in the second filial generation, for which reason the weight of this generation should correspond rather more to the purely intermediate weight. CASTLE regards it as most probable that the genetic factors determining size are general in their effect. It will be seen that he is not in agreement with WRIGHT (1918), who infers from the data given by MACDOWELL and CASTLE that these factors influence to some extent both certain groups of body parts and some skeletal parts. In later work by WRIGHT (1932), computations are made of the proportion of the variation which can be ascribed to general factors, group, and special factors. From these computations it appears that about 84 per cent of the variation in the differences of weight must be considered due to general factors, whereas only 6 and 10 per cent can be ascribed to group factors and special factors respectively. This is true only if it can be presumed that the experimental material has, from a genetical point of view, been so completely homogeneous that all, or by far the greater part, of the variance in the F_1-generation is environmentally induced.

Attempts to compute how many genes that influence the inheritance of size (CASTLE 1921, 1922), must be considered a failure. The only conclusion which may justifiably be drawn from the estimates which have been made is that in all probability there are many chromosomes or linkage groups containing size genes.

All the investigations into the problem of size inheritance referred to have been concerned with the differences in the size of adult animals, but a few have touched on the difference in growth within the various breeds on which experiments have been made. Besides using the body weight as a criterion, it has here been possible to use direct skeletal measurements to express differences in size. However, if investigations are concerned with the inheritance of size in newborn individuals, it is impossible to find any other adequate criteria than the weight. A chemical comparison (GREGORY and GOSS 1933) or a histological examination are possibilities which will be taken up for discussion at a later stage, but as these methods are only applicable to stillborn fetuses, they are of no use for the purpose of continued investigations into the post-natal influence.

Direct experiments concerned with size differences in newborn rabbits have only been made to a very limited extent. In 1922 KOPEĆ published the results

VOL. 31 1950

of a physiologico-genetical investigation into the effect of superfecundation in rabbits, raising the question whether fetuses by different sires have a reciprocal influence, a unilateral influence, or develop independently of each other. There is only a purely technical difference between his investigations and HEAPE's epoch-making experiments (1890, 1897) with transplantation of fertilized ova from one breed to another. HEAPE did not submit his limited material to any detailed examination but merely stated that two Angora young, borne by a Flemish Giant, were bigger and more vigorous than any of the other young of the same litter, viz. four Flemish Giant, and that they retained this superiority. He writes: "The experiment described above was undertaken to determine in the first place what effect, if any, a uterine foster-mother would have upon her foster-children, and whether or not the presence and development of foreign ova in the uterus of a mother would affect the offspring of that mother born at the same time. So far as this single case goes, the evidence is negative."

Unfortunately it is not possible to decide whether he is exclusively referring to purely qualitative characters or if his statements are meant to refer to size also. The latter does not seem to be the case, for his conclusion (of 1897) contains *inter alia* the following passage: "... that the uterine foster-mother exerts no modifying influence upon her foster-children in so far as can be tested by the examination of a single generation."

KOPEĆ (1922, 1923 b) used in his experiment two breeds of rabbits with significant differences in the birth weight as well as in the adult weight. The lighter race, the Himalaya, was used as the mother race, and in three cases superfecundation was successfully carried out, with the result that in the same litter were born purebred Himalaya young and crossbred young by Himalaya ♀ and Silver ♂, the heavier of the breeds. The results of the experiment are collected in the table below:

Normal Mating:	Litter Size	Mean Weight	Standard Deviation	Number
A. Himalaya ♀ × Himalaya ♂	5.0	34.00 ± 0.43	4.74	56
B. „ ♀ × Silver ♂	4.3	40.81 ± 0.46	4.44	43
Superfecundation:				
C. Himalaya ♀ × Himalaya ♂	5.7	36.30 ± 0.81	3.80	10
D. „ ♀ × Silver ♂	—	45.57 ± 1.18	4.61	7

By comparing the weight of the offspring of the two normally mated groups, a difference of 6.81 ± 0.63 gms. is found, and by comparing the two superfecundation groups, the difference is 9.27 ± 1.43 gms. This means that there is an actual difference between the races, even if the development takes place in the same uterine environment. KOPEĆ further takes it for granted that the pre-

sence of fetuses of different genotypes exerts a reciprocal positive influence, without, however, being able to show any significant difference (2.30 ± 0.92 gms.) between Himalaya fetuses born according to one or the other mating system. He based his supposition on the difference in the size of the litters, for if they were the same, there should be a somewhat greater weight for Himalayas born in the heterogeneous litters. It is probable that a tested litter size of 6 in a mixed litter by a Himalaya mother corresponds to a "biological" litter size that is greater, or expressed in other words, that the computed average litter size of 5.7 corresponds to a litter size of 6.9 in the case of purebred Himalaya. On the other hand, the difference between the Himalaya and Silver crosses according to the two mating systems is significant (4.76 ± 1.27 gms.), just as was the difference between Himalaya and the crosses, both in the case of single and double mating. Incidentally, no after-effect was traceable in the second generation of Himalaya, all the offspring having a considerably higher birth weight than the young of the first generation.

In a later investigation, KOPEĆ (1923 a, 1924 b) gives more exact results of the cross-experiments with Himalaya and Silver. The tested pure breeds had an adult weight which in the case of Himalaya ranged from 1675 to 2015 gms., the average being 1831 gms., and for Silver the weight ranged from 1991 to 2518 gms., with an average of 2286 gms. In order to reduce the influence due to different numbers of young in the litters, several of the calculations are restricted to litter sizes of four, five, and six. The computed averages are shown in the table below:

	Litter Size	Mean Birth Weight	Standard Deviation	Number
Pure Breeds:				
Himalaya ♀ × Himalaya ♂	4.8	35.92 ± 0.33	5.73	139
Silver ♀ × Silver ♂	4.8	44.19 ± 0.34	5.08	101
F_1-Generation:				
Himalaya ♀ × Silver ♂	4.6	41.91 ± 0.37	5.50	102
Silver ♀ × Himalaya ♂	4.9	43.30 ± 0.39	4.60	63
F_2-Generation by:				
Himalaya ♀ × Silver ♂	5.2	41.06 ± 0.37	7.68	198
Silver ♀ × Himalaya ♂	4.8	41.26 ± 0.60	8.94	101
F_2-Generation, Himalaya-Coloured Offspring:				
Himalaya ♀ × Silver ♂	5.2	41.42 ± 0.68	7.22	51
Silver ♀ × Himalaya ♂	4.7	42.16 ± 0.91	7.38	30

VOL. 31 1950

It appears from this table that the calculated mean weight of the F_1-generation ranges between the weights of the two pure breeds, but in both cross-groups the weight is seen to be somewhat above the weight to be expected on purely theoretical grounds. There is no significant birth weight difference between the offspring of Silver ♀ × Himalaya ♂ and the offspring of purebred Silver, as far as the averages are concerned.

In the case of the individual mothers, the difference in weight between purebred offspring and crossbred offspring is significant. In two instances the weight of crossbred offspring is higher than that of purebred offspring and in two instances lower. A fact which should be emphasized is that, in spite of irregularities, the cross-experiments reveal that the highest birth weights are found in young borne by mothers of the larger race. The difference is apparently significant ($P < 0.01$).

The F_2-generation only differs from the F_1 through greater diversity, and the maternal influence that is shown to exist in F_1 seems to disappear in the following generation. Furthermore, KOPEĆ examined Himalaya-coloured individuals of the F_2-generation, but found no difference whatever between their birth weight and the birth weight of the other offspring of the same generation. As far as this colour marking is concerned, there is ample proof that it is inherited quite independently of size, but this does not by any means exclude the possibility that certain size genes in rabbits are coupled with other genes which determine colour or colour markings, as has been shown to be the case in mice and rats.

When examining the inheritance of size it is necessary that an attempt should be made to elucidate how closely related the size at birth is to the adult size, in order to decide whether it is possible on the basis of our knowledge of birth weights to draw conclusions of more general importance to the problem as a whole. KOPEĆ (1925, 1926) calculated the correlation between the birth weight and the weight at a certain age on the basis of data divided, according to the birth weight, into five groups ranging between 31 and 60 gms., irrespective of the litter size, which appeared to be fairly even within the individual groups. Consequently this division would correspond to a genetically caused difference in size. The weight of the animals in the group with the higher birth weight was higher during the whole growth period. The conclusion of these experiments is expressed as follows: The genetically determined weight at birth can be used as a criterion for the study of the inheritance of size, but it is an absolute condition that the influence of the environmental factors is known and controlled.

It must be admitted that most of the early investigations into the problem of the inheritance of weight in rabbits have been made with a comparatively small number of individuals as experimental material, and consequently it is not possible to estimate with any great certainty the actual diversity in the F_1-

and the P-generation. In a few cases also the F_2-generation has been examined, but generally the number of the individuals in the tests has been too small to expect the appearance of types having the same weight as the two original races, if it is presumed that size is determined by a number of genes with additive effect. This applies for example to the investigations in which Polish and Flemish Giant have been used as testing material (PUNNETT and BAILEY 1918, CASTLE 1922). In the experiments in which two pure breeds of almost equal body weight are used, as e.g. Polish and Himalaya (CASTLE 1922, KOPEĆ 1924) complete segregation is found, with the result that in the F_2-generation there are individuals weighing just as little as the smaller of the parental breeds, and individuals weighing just as much or more than the heavier of the two original breeds. PEASE (1928) noted this fact and has criticized the experiments. In an introduction to his own experiments, he writes that if a large number of animals are tested after all precautions have been taken and it is yet impossible to prove the existence of individuals of the F_2-generation which, in so far as size is concerned, are equal to the two original breeds, this fact would in itself be of fundamental genetical interest; but if on the other hand an F_2-generation displays "the complete range", this will be proof that the general Mendelian interpretation of multiple factors applies to the inheritance of weight in mammals.

VOL. 31 1950

By using PUNNETT and BAILEY's stocks as his material, PEASE (1928) succeeded in proving that the F_2-generation did segregate so completely that all intermediates between the two parental types were represented, but the individual F_1-animals bred very differently. A few had progeny whose weight was equal to the weight of the F_1-generation or ranged between the F_1 and the lighter of the breeds of the P-generation. PEASE finds in this fact the explanation of the results arrived at by CASTLE (1922) and PUNNETT and BAILEY (1918).

In the investigations referred to above there are, as mentioned, certain divergences of opinion with regard to the weight of the F_1-generation. The writer holds the view that these are partly explained by the fact that CASTLE has not used full-grown Flemish Giant (cf. CASTLE 1922, table 1, table 3, and fig. 5), nor did he pay any attention to whether the cross was between Large breed ♂ and Polish ♀, or *vice versa*. Further, the conflicting results are in all probability partly due to the fact that the crosses between Flemish Giant and Polish in PUNNETT and BAILEY's experiments (1918) and PEASE's experiments (1928) are reciprocal. As previously mentioned, the experiments made at The Animal Breeding Institute revealed, with regard to the body weight, very great differences between the reciprocal crosses. The mean weight of 71 F_1-crosses between Large breed ♂ and Polish ♀ was 2.257 ± 0.042 kgms., whereas the mean weight of 69 animals of the cross Polish ♂ × Large breed ♀ was 2.744 ± 0.032 kgms. The difference of 0.487 kgms. is significant

($P < 0.001$). These means refer to all offspring of the two crosses which lived to the age of ten months.

In more recent investigations, CASTLE (1929, 1931, 1934 a) found, by back-crossing F_1-individuals with the two parental breeds, distinct intermediate body size in the three-quarter-blood generations. The object of these experiments was to examine whether size genes might possibly be linked to genes determining colour or special colour markings, as has been observed in mice (cf. below), but the result of the experiments was in that respect entirely negative and therefore in complete accordance with KOPEĆ's conclusions (1924 b). The variation proved to be greater in the back-cross generations than in the F_1-generations, but there was nothing to indicate that the offspring grouped themselves into a greater or smaller number of classes, for the variation was continuous. CASTLE himself considered this clear evidence of a genetic segregation of the factors determining size, and gave as his opinion that, providing the interpretation of multiple factors is accepted, it must be considered proved that many genes act as determinants of the differences in size between large and small breeds.

Of great interest to the entire study of the factors—genetic and environmental—which influence size is the observed divergence between reciprocal crosses between breeds which vary in either birth weight or adult weight.

Without taking the modifying effect of the size of the litter into consideration CASTLE (1929) gave the mean weight of newborn Polish as 35.0 gms., of newborn offspring by Flemish Giant ♂ and Polish ♀ as 55.3 gms., of young by the reciprocal cross as 59.4 gms., and as 62.6 gms. in the case of offspring by Flemish Giant. It is probable that allowance for the litter size would not only emphasize the difference between small and large breeds, but would also cause the differences between the reciprocal crosses to stand out more prominently. In addition, a clearer picture would be given of the possible existence of purely intermediate inheritance and at the same time enable us to observe the effects of heterosis, if any. It is generally the case that the size of the litters by Flemish Giant ♀♀ is numerically twice as large as the litters by Polish ♀♀. CASTLE himself observed the difference in size but did not consider it to be of a lasting nature. He stated, however, that it was clearly present until the age of approximately two months. More recently CASTLE (1934 b) carried out some experiments in order to find out whether the difference in crossbred offspring by reciprocal crosses is lasting and significant or not. In these experiments he used New Zealand White as the large breed and both Polish and Himalaya as the small breed. Unfortunately no figures for the birth weight or the adult weight were given, only the weight at the age of four months being published, and in agreement with the experimental results previously referred to, statistical analysis showed a difference between the reciprocal F_1-generations. CASTLE concluded that there must be something in addition to

the chromosal genes which acts as a co-determinant of the size of crosses between breeds differing in size. If this co-determinant exists already in the fertilized egg it is probably a cytoplasmic influence (CASTLE 1933), but if the difference in weight is caused by non-heritable factors it is most likely due to "agencies" operating during the pregnancy. It was not possible for CASTLE to separate the genetic and the non-genetic influence.

A special case of size inheritance has been described by GREENE *et al.* (1934) and GREENE (1940), who showed that dwarfism in a stock of Polish rabbits was determined by a single recessive gene, which also exerts an influence in heterozygous animals. The homozygous lethal dwarfs are one third the size of the normal at birth, while the heterozygous animals are about two thirds of the normal litter mates. The weight of the dwarfs is 15 gms. on an average. It is conceived, "that the lethal dwarfs represents the final stage in a series of size mutations leading to distinct classes of individuals whose size is of a measured order of reduction in relation to their forebears." In the material presented in this paper the same range in birth weight as in GREENE's material, i.e. from 15 gms. to 63 gms., has been observed. However, the distribution of the birth weight is perfectly normal for 760 Polish young, following the function $y = \frac{760 \cdot 3}{7.66\sqrt{2\pi}} e^{-\frac{(x-39.55)^2}{2 \cdot 7.66^2}}$, and thus there is nothing to indicate that the gene for dwarfism ever has been introduced in the stock used.

In this connection it is of interest to draw attention to an investigation by PAINTER (1928) into the problem of cell size and body size in rabbits. In this case also Flemish Giant and Polish as well as crosses between them were used. It was not possible to show any difference in the size of the ova of the pure breeds, but already in 9 days old embryos there was a tendency towards the cells being larger in the hybrids than in the pure races. In 12 days old embryos the number of cells in Polish embryos appeared to be significantly less than the number of cells in Flemish Giant and cross embryos, and he supposed the difference in the embryo sizes to be due to a quicker cell division in the large races. This more rapid division he presumed to be genetically determined. This is in accordance with CASTLE and GREGORY (1929) and GREGORY (1930), who showed a difference in the number of blastomeres between Polish and "3/4" Large race at the age of 48 hours, i.e. the time when the blastocysts enter the morula stage. The time to this stage varied from 41 to 75 hours, but was reached earlier by the larger breed than by Polish. At earlier stages no difference could be ascertained. CASTLE and GREGORY (1929) found that, at the age of 100 hours, Large race was less developed than "7/8" Polish, but they presumed the ova of Large race to have been fertilized later than the ova of Polish. In the case of the Polish breed the diameter of the blastodermic vesicle was twice that of Flemish Giant, 31.4 and 16.3 millimetres respectively (by 50 times

VOL. 31 1950

magnification). A little later (116 hours), no difference in cell size was evident, but at 144 hours a difference had developed which could probably be attributed to more rapid cell divisions, for the size of the cells was the same in both cases.

OTHER MAMMALS.

Direct investigations into the problem of the genetic part of the variation in the birth weight have been made to a somewhat larger extent in the case of other animals than the rabbit. In the main, the material of the investigations has been smaller multiparous animals, and it applies to several of these experiments as in the case of those made with rabbits, that the modifying influence on the birth weight of such a strong factor as the litter size is not always given due consideration. As far as larger farm animals are concerned, investigations into the inheritance of birth weight are based rather on a statistical treatment of the collected data.

In his experiments made with mice, VETULANI (1931) found, by crossing small breed female with large breed male, purely intermediate inheritance of the birth weight, whereas the reciprocal cross showed the effect of either heterosis or maternal influence. It is probably in both cases a question of interaction of heterosis and maternal influence, but in the former cross the maternal influence is counteracted by the increasing effect of heterosis on the birth weight, with the result that the ascertained mean weight of the newborn mice is identical with the mean of the two pure breeds. In the reverse cross, i.e. between large breed female and small breed male, the effect of heterosis and the maternal influence are cumulative, resulting in the weight of the young being approximately the same as in the case of large breed. In the latter experiment there was a slight difference in the litter size, which, however, did not seem to be of any particular importance. In agreement with similar crosses between rabbits, VETULANI found a decrease in the coefficients of variation from the P- to the F_1-generation and a somewhat greater variation in the F_2-generation. This is strong evidence that in mice also the birth weight is determined by a number of genes with additive effect. In an initial experiment with mice, GREEN (1931) was unable to show any difference in the reciprocal crosses between parental breeds of greatly varying sizes. The weight of the young (less than 24 hours old) were in both cases nearer to the weight of the larger breed than to the intermediate weight. By comparing the birth weights with the weights of the adult animals it is evident that here too the size inheritance can be explained by the hypothesis of multiple factors, but in this particular instance the special effect of some genes cannot be disregarded. In a more recent investigation (GREEN 1935 a) a significant difference was found between females of reciprocal crosses. MARSHAK's investigations (1934, 1936) revealed a heavier

birth weight for cross offspring than for the pure races, but at the same time he observed a maternal influence on the birth weight in the case of male progeny from a cross between small race male and large race female. As far as the birth weight is concerned, the difference between the two races is very small, the average birth weight of males of the A-strain being 1.42 ± 0.020 gms. ($n = 60$), that of the B-strain 1.43 ± 0.018 gms. ($n = 56$), whereas the females of both strains had a birth weight of 1.37 gms. In the F_1-generation the birth weight of females by reciprocal crosses was once more the same, viz. 1.43 gms., whereas the weight of the males was: B ♀ × A ♂ 1.57 ± 0.038 gms. ($n = 19$) and A ♀ × B ♂ 1.47 ± 0.026 gms. ($n = 28$). In these investigations an environmentally caused maternal influence must be disregarded. If any real importance is attached to the difference between the two groups (it being only twice as great as the standard error) the maternal effect is presumably due to cytoplasmic inheritance.

In investigations into the question of size in mice and rats, the special effect of individual genes must frequently be taken into consideration. Thus for instance it has been proved that the genes for "brown" and "dilution" have an increasing effect on the body weight (GREEN 1935 b, FELDMAN 1935, CASTLE *et al.* 1936 a & b). It is not possible to enter into a more detailed account of the positive or negative influence upon the body size of the various mutating genes, but reference should be made to recent work by LAW (1938), CASTLE (1941), and others who have shown that several genes for colour, besides the two mentioned above, have either an increasing or a decreasing effect on the body weight. Moreover, in contrast to the results of the investigations into linkage of colour genes and genes for body size in rabbits (KOPEĆ 1924, CASTLE 1929), KEELER (1947) succeeded in proving the special effect of some particular genes in mice and rats. It is still an open question whether all size differences are attributable to chromosomal genes (cf. GRÜNEBERG 1943), but none of the investigations referred to immediately above have thrown any light on the question whether these genes have any measurable influence on the size of the young at birth, or whether the effect only manifests itself in the adult animals.

In experiments with guinea pigs, WRIGHT (1922) found "that prenatal rate of growth depends largely on the characteristics of the dam as far as it depends on heredity at all." By crossing inbred strains he observed that the birth weight of the second cross-generation was about 9 per cent above the average of the inbred strains. He attributed three quarters of the influence to the mothers, but only one quarter to the young. Further investigations into the inheritance of the birth weight in guinea pigs were made by EATON (1932), who concluded that about 47 per cent of the variation was genetically determined.

In a cross experiment between a wild species of guinea pigs (*Cavia rufescens*) and a domesticated species (*Cavia porcellus*) UBISCH and MELLO

VOL. 31 1950

(1940) reached conclusions regarding the inheritance of birth weight which differ somewhat from what has generally been drawn. In the cross between wild ♀ (small ♀) and domesticated ♂ (large ♂), the weight of the young at birth was the same as that of the wild species (possibly slightly less), whereas the reciprocal cross between large race domesticated dam and small race wild sire yielded offspring whose weight at birth ranged far above the normal birth weight of domesticated guinea pigs. Unfortunately, because of the limited amount of material, it is not possible to decide whether in this instance special circumstances may be presumed to have influenced a species-cross, or whether the result can be attributed to accidental deviations. The results are in agreement with DETLEFSEN's (1914) data for the weight of ten days old offspring.

CHAPMAN and LUSH (1932) examined a sheep population and found that, on the supposition of inheritance being additive, at the most, 25 to 30 per cent of the variance in the birth weight was attributable to hereditary factors. Further, they showed that the sire's influence on the birth weight could be estimated at approximately 7.5 per cent of the total variance. This conclusion is quite in keeping with the result of an investigation made by KINCAID (1943), in which it was claimed that the total variance in the birth weight of lambs was reduced by 7.8 per cent on subtraction of the part attributable to differences between groups of rams of two different breeds.

LUSH *et al.* (1934) studied the individual sources of variation influencing the birth weight of pigs. The conclusion was reached that 3 per cent of the variance "in birth weight is due to differences in the genes possessed by the pigs themselves." Only two per cent of the variance was between "breeds", because the material on which the calculations were based had not been selected with "conscious attention to birth weight and without any attempts to secure contrasts in adult size." Finally a "rough apportionment of the variance due to various causes" is made: "Heredity of the pigs ... 6 per cent, Environment common to litter-mates ... 47 per cent and Environment not common to litter-mates ... 47 per cent."

Investigations into the inheritance of the birth weight of uniparous animals are less numerous. TYLER *et al.* (1947) calculated for Holstein-Friesian cattle that 60—70 per cent of the variation in the weight of calves at birth within herds, sex, and calving number was due to genetic dissimilarities between calves. In the case of Red Danish cattle and Swedish Red and White cattle at the most only 12 per cent of the total variation in the weight of calves seems to be genetically determined (VENGE 1949).

WALTON and HAMMOND (1938) crossed Shetland ponies with Shire horses and obtained results which are similar to those described for the cross between two species of guinea pigs. They are shown in the table below (the average figures being computed by the writer):

Parental breeds:	Weight of the offspring, kgms.	Birth weight in per cent of the weight of the dam
Shetland ♀ × Shetland ♂	19.5	9.4
— × —	18.2	12.8
— × —	22.2	12.0
— × —	18.6	8.1
(Average	19.6)	
Shetland ♀ × Shire ♂	17.2	7.5
— × —	13.6	6.0
— × —	22.7	12.2
(Average	17.8)	
Shire ♀ × Shetland ♂	53.5	6.7
— × —	45.3	5.7
(Average	49.4)	
Shire ♀ × Shire ♂	59.4	7.4
— × —	82.6	10.2
(Average	71.0)	

The birth weight of crosses between Shetland mare and Shire stallion is equal to the weight of pure Shetland offspring (or slightly less), whereas the reciprocal cross produces offspring whose birth weight is more than two and a half times larger. If a birth weight of 70 to 75 kgms. (68—77) is regarded as the normal for foals of pure Shire, the weight of the offspring by Shetland ♂ × × Shire ♀ is slightly above the intermediate, and in all probability this is due to purely maternal influence. Assuming the absence of special genes, the greatly decreased birth weight of the Shetland ♀ × Shire ♂ crosses must be the result of the combined effect of purely maternal influence operating through the intrauterine environment, and a possible cytoplasmic inheritance.

*

From the preceding review it is thus evident that in spite of the many investigations which have been made into the inheritance of birth weight, research workers have not succeded in establishing with certainty the hypothesis of the effect of multiple genes. The only point on which agreement has been reached is that birth weight is to a very large extent subject to environmental influences, and it is exceedingly difficult, even with experiments on smaller animals, to ascertain the extent of the modifying effect of these environmental factors. It is true of these experiments, as in investigations on the genetics of populations, that the collection of data frequently extends over several years, whereby new sources of variations are added to the material. Further, in the case of multiparous animals, the birth weight is influenced by litter size, length of gestation period, size of mother etc., matters which will be taken up for discussion in a subsequent chapter.

VOL
31
195

The best proof of the hypothesis of the effect of multiple genes on size has come from experiments in which crosses have been made between species and between breeds of widely differing size. In more special cases where lethal factors are involved size is found to be determined by one single Mendelian factor. For the normal development of size, however, other systems apply; the influence of dominance, epistasy and other genetical phenomena is still an unsolved problem, partly because a gene analysis is impossible to carry through. A reason for the diversity of the results obtained is that it has been impossible to separate the effects of heterosis, the maternal influence, and purely cytoplasmic influence from each other, because the F_1-generation is always borne by the P-generation, the F_2-generation by the F_1-generation etc. The effects of these three factors are therefore always inseparably mixed in the first filial generation and persist, though progressively less marked, in the subsequent generations. Consequently it is necessary to resort to methods which will enable the separation of the various environmentally caused sources of variations from the variation which must be presumed to be of genetic origin. By transplanting fertilized ova F_1-mothers may be induced to bear F_1-fetuses, or even to bear fetuses belonging to the P-generation (pure breeds). By the use of this technique it is possible, as will be subsequently explained, to separate the influence of these various factors.

VOL. 31 1950

MATERIAL AND METHODS OF THE PRESENT INVESTIGATION.

In the present work, the material which has been used is normal pure breeds, normal crosses between pure breeds and in addition transplantations of fertilized ova between the two breeds and crosses used according to the following plan:

Transplantations of fertilized ova from:		*Transplanted to dam of:*
A. Large race ♀ × Large race ♂	.....	Small race
B. Small race ♀ × Small race ♂	.....	Large race
C. Large race ♀ × Small race ♂	.....	Small race
D. Small race ♀ × Large race ♂	.....	Large race
E. Large race ♀ × Large race ♂ Small race ♀ × Small race ♂	simultaneously	Intermediate size
F. Large race ♀ × Small race ♂ Small race ♀ × Large race ♂	simultaneously	Intermediate size

By a combination of the data from the different transplantation groups or by a comparison of these data with the results of the ordinary or normal groups of pure breeds and crosses, it will be possible by dividing up the sources of variation, to assess the extent to which size is determined by hereditary factors

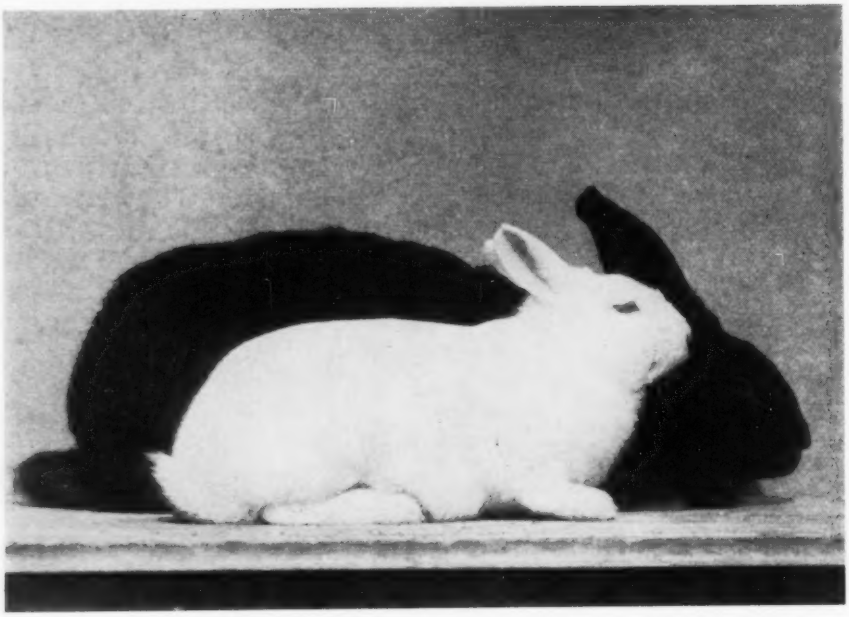

Fig. 2. A Flemish Giant doe borne by Polish, and a Polish doe borne by Flemish Giant. Age 11 months. (Photo. I. DYRENDAHL.)

and likewise the extent of the modifying influence of environmental factors. As most probably the individual environmental factors exert an unequally great influence on the birth weight within the different groups, the influence of some of these factors will be subjected to an examination first.

For the sake of simplification the designation "*Normal*" will be used in the following of offspring born after development of the fetus in its own mother (or in a foster-mother of the same breed), whereas offspring born after development of the fetus in a foster-mother not of the same breed will be termed "*Transplanted*". Further, *L* will be used for *Large race*, *S* for *Small race*, *LS* refers to crosses between Large race buck and Small race doe, while *SL* symbolises the crosses between Small race buck and Large race doe. The different groups will be designated A, B, C etc. with an index "I" for Normal and "II" for Transplanted.

MATERIAL.

Animals. The rabbit breeds forming the material of this investigation are largely the same as were used in several of the previously mentioned experiments, i.e. breeds differing so much in size when full-grown that the heavier has a weight that is about three times the weight of the smaller breed (Fig. 2).

Polish, whose adult weight is about 1 1/2 kgms., was used as the Small race. The average weight of the stock has varied somewhat during the experimental period which lasted from 1938 to 1949, principally due to the fact that new individuals had to be purchased rather frequently, and it has not always been possible to obtain sufficiently small animals. On the other hand, some inbreeding has taken place during the initial stages of the building up of the population, resulting in a slight decrease of the body weight. On the cessation of this inbreeding, the average weight of the population increased once more, and there have been instances of individuals weighing more than 2 kgms. During the last few years outbreeding has been used as extensively as possible within the pure breeds.

For the experimental period as a whole the mean weight of all the Polish rabbits is 1.657 ±0.016 kgms. at the age of ten months. Particularly during the last two or tree years an increase in the weight has taken place, but it is doubtful whether this increase in the mean weight is perceptibly influenced by the Polish rabbits born after transplantations to Large race or crosses. There does not seem to be any significant difference between normally born and transplanted individuals. On the other hand, the difference between the two sexes, based on the weights of animals classed into age groups of one month interval, is significant ($P < 0.01$); further, the Polish breed seems to be subject to a seasonal influence resulting in a somewhat higher weight of the adult animals during the Winter months than during the Spring and Summer months, i.e. during the breeding season proper. The means as well as the standard deviation are seen in Table 1. Figure 3 illustrates graphically how the weights of the adult individuals vary about the mean.

During the first few years of the experimental period the breed used as the Large race was Flemish Giant, which under favourable conditions can reach a weight of five or six kgms. or more, but in a large population where individual care and attention is too expensive, it rarely happens that the weight of the animals exceeds six kgms. From the point of view of reproduction this race is somewhat more difficult to handle than the Polish rabbit. Although on an average Flemish Giant bear a considerably greater number of young than Polish, the litter size of the former is subject to far greater variation than is observed in the case of the latter. In addition, the weight of the Flemish Giant offspring tends to be rather more variable.

For a short period a few males of the Chinchilla breed were used for purposes of incrossing into the population of Flemish Giant, and since the Spring of 1946 Vienna Blue have been used extensively, either as pure breed or crossed with Flemish Giant. Partly by breeding the animals pure, and partly by crossbreeding them, with subsequent backcrossing to the pure breeds, it has proved possible to improve the constitution of the Large race animals considerably. One consequence of this has been that it is usually very much easier to make

the animals pregnant. During the last few years the crossbreeding between the large breeds takes the form of selective crossing based on morphological characters, wild-coloured and black individuals always being mated to pure Flemish Giant, and blue-coloured individuals being mated back to pure Vienna Blue. After having been backcrossed through four generations the females are again regarded as purebred.

The mean weight of all Large race individuals is 4.301 ± 0.054 kgms. at the age of 12 months (Table 1). The standard deviation is somewhat larger than is the case with the other groups, but in relation to the mean there is no difference between the groups. The coefficient of variation is 14.93 per cent in Large race and 15.05 per cent in Polish. As far as Large race is concerned there is a significant difference between males and females ($P < 0.01$), and further the season affects the weight of the animals ($P < 0.01$), probably due to difference in fatness.

For purposes of comparison, the mean weights of individuals out of reci-

TABLE 1. Mean body weight of the different breeds.

Breed and sex	Number of animals	Mean weight, kgms.	Standard deviation, kgms.	Coefficient of variation, %
Polish				
♂♂	83	1.59	0.243	15.32
♀♀	161	1.69	0.246	14.53
Total	244	1.66	0.249	15.05
Large ♂ × Polish ♀				
♂♂	25	2.19	0.312	14.24
♀♀	30	2.35	0.437	18.60
Total	55	2.28	0.390	17.11
Polish ♂ × Large ♀				
♂♂	12	2.67	0.232	8.70
♀♀	36	2.88	0.411	14.26
Total	48	2.83	0.384	13.58
Large race				
♂♂	30	3.98	0.486	12.22
♀♀	111	4.39	0.653	14.89
Total	141	4.30	0.642	14.93

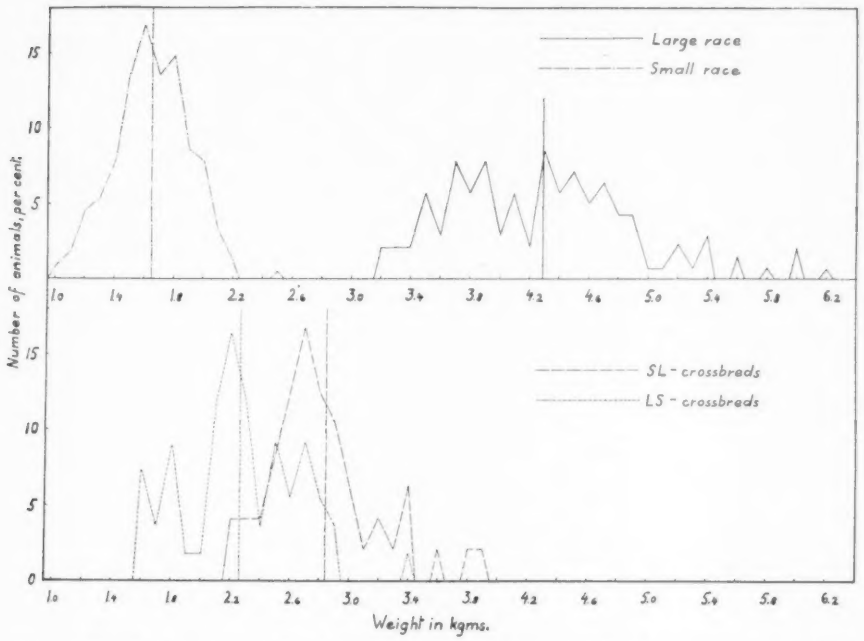

Fig. 3. Distribution of weight about the mean for pure breeds and reciprocal crossbreds.

procal crosses between the two pure races are recorded in Table 1. As previously mentioned, there is a statistically significant difference between crosses borne by Large race mother and those borne by Polish mother ($P < 0.001$). The mean weight of the former has been 2.830 ± 0.056 kgms. at the age of 11 months, whereas the average weight at the same age has been 2.277 ± 0.053 kgms. in the case of crosses of Large race buck × Small race doe. In both groups the males are somewhat lighter than the females, but due to the comparatively small number of animals on which the calculations are based the difference is not significant. It should be pointed out, however, that the coefficient of variation is not smaller in these F_1-generations than in the P-generations.

Feeding. The animals have been fed twice a day except on Sundays, when they have been fed once. Calculations have not been made of the feed requirements of the individual animals, but abundant quantities of fodder have always been available on the grounds that feeding *ad libitum* would make it possible to exploit the growing power of the animal to the full. The roughage given in the Summer has consisted of mixed grasses, and in the Winter of clover and timothy hay, swedes an other turnips, carrots, and dried sugar beet pulp. In addition, concentrates consisting of 25 per cent wheat bran and 75 per cent

oats and barley have been given all the year round. During the suckling period skim milk has been given to the mothers, and the young have been given skim milk until they were 8 weeks old (weaning time has been six weeks after birth). All the animals have been given additional minerals with the concentrates, and during the Winter months the concentrates have received an admixture of cod liver oil.

Health. On the whole, the state of health of the animals has been excellent. No cases of coccidiosis have been observed, but the purchased animals have caused scattered cases of spirochactosis and ear mites to occur. The affected animals have been rapidly treated, and only in a couple of cases has it been necessary to kill the diseased individuals. In the Spring of 1949, however, a rather severe epizootic of snuffles suddenly broke out. By killing the affected males and by isolating and treating the diseased females and young, it was possible to keep the disease in check, but there is no doubt that it has had an adverse effect on the transplantation results. A detailed account of this will be given later on.

VOL.
31
195

METHODS.

General conditions. Since the investigations were started in the Spring of 1938 they have been carried on with a view to determining the size of the hereditary variation in so far as both the birth weight and the adult weight are concerned. Accordingly all the animals have been weighed regularly from as soon as possible after birth until the adult size has been reached, the weighings taking place at regular intervals of eight to fourteen days in the case of the youngest animals, and one month in the case of the older animals (> 3 mos.). Unfortunately it has not been practicable, for reasons of economy, to watch the births, and owing to this there is a variation in the birth weight, the size of which is unknown. As working hours have been from 8.00 a. m. to 4.30 p. m. it is possible for as long as 15 1/2 hours to elapse between birth and weighing. However, as most births take place early in the morning and the young do not as a rule take any nourishment on the first day of their life (Kopeć 1923 c, 1924 a), it is permissible to ignore this error.

From Table 1 in preceding section it appeared that the weight of Polish at the age of 10 months, the weight of the crosses at 11 months, and that of Large race at the age of 12 months were to be considered representative of the adult weights, for at these respective ages all the animals are regarded as being fully grown. A study of the weight charts of the individual animals will show that at these ages they have passed "the turning point" or "maximum point" of their weight curves (Pease 1928) although these points have not always been observable.

Simultaneously with the weighing of the newly born animals they have been

marked by means of clips in the ears. At weaning, the litter number has been tattooed into the left ear. The weights of the young are stated in grammes (gms.), and in the case of the adult animals, the figures are given in kilos (kgms.) to two decimal places. Further, it must be noted that the calculations are based on the weights of every individual, no division into groups having been made. During the later years, the sex of all animals has been determined at birth.

In the statistical treatment of the data, reference books by BONNIER—TEDIN (1940), SNEDECOR (1946), YULE and KENDALL (1947), and HALD (1948) have been used.

The technique of transplantation of fertilized ova. A very important part of this work concerns the birth weight of young born after transplantations of fertilized ova. Therefore a brief survey of the method used in making the transplantations will be given.

As is well known, the rabbit belongs to the group of mammals having non-spontaneous ovulation, i.e. the last part of the follicle growth does not start and the follicle rupture does not take place until after copulation. The ovulation takes place ten to ten and a half hours after copulation (HAMMOND and MARSHALL 1925, WALTON and HAMMOND 1928 and others), sometimes slightly earlier or later. Normally, after the ovulation, a *corpus luteum* fills each ruptured follicle, and if no pregnancy occurs, these corpora lutea cause a pseudo-pregnancy that lasts sixteen to seventeen days after the copulation. After mating to sterile (vasectomized) bucks, the does will ovulate in the normal way, and by subsequent transplantation of fertilized ova to the Fallopian tube or uterus (BERNSTEIN 1938) it is possible to bring about normal pregnancy.

The first transplantations of ova from one rabbit to another were carried out by HEAPE (1890), who used a Flemish Giant doe as receptor and an Angora doe as donor. The donor had been mated thirty-two hours before it was killed whereas the receptor was mated as late as three hours before the transplantation took place. In his first experiment HEAPE succeeded in transplanting two fertilized ova at the four cell stage, and after normal gestation the receptor bore, among others, two Angora young. Later HEAPE (1897), repeated the experiments with similar success.

Some forty years later PINCUS and ENZMANN (1934) resumed the work of transplanting fertilized ova from one breed of rabbits to another. In one case ten ova were collected from the Fallopian tube of a doe that had been mated to a vasectomized buck thirteen hours previously. These ova were fertilized *in vitro* and were subsequently transplanted to a doe that had been mated to a vasectomized buck forty-eight hours earlier, and after thirty-three days seven young were born. In another case five fertilized ova were transplanted after having been stored for twenty-four hours in a Carrel flask, and thirty-one days later two young were born.

Later, PINCUS concentrated rather on a study of the activation of unfertilized ova, continuing, however, his transplantation experiments (1939 a & b, 1940). In an earlier work (1930) he explains the technique used by him when collecting the ova from the Fallopian tube. In all essentials this technique is identical with the one used in these present experiments, as will be seen from the following detailed description.

The experimental does, hereafter called *Donor* and *Receptor,* are simultaneously mated to fertile and vasectomized bucks respectively. In consequence of this, it is possible to adhere more closely to the normal sequence of events which follow copulation than has been the case in some earlier investigations, e.g. HEAPE (1890) and PINCUS and ENZMANN (1934) in their first experiments. Twenty-two to twenty-four hours after the mating, somewhat less during the first years experiments, the donor is killed, after which the Fallopian tubes are extracted and released as quickly as possible from any attached mesentery. It has been necessary to remove these membranes, for in the first place the washing out of the Fallopian tubes is thereby facilitated and further it is easier to prevent fatty substance from forming beads on the surface of the washing liquid. If these measures are not taken the detection of the ova is apt to be rather difficult. While the second tub is being extracted the first is stored in a physiological salt solution (Ringer's solution: 6.5 gms. NaCl, 0.14 gms. KCl, 0.20 gms. $NaHCO_3$, 0.12 gms. $CaCl_2$, 0.01 gms. NaH_2PO_4, ad 1000 cc H_2O) at a temperature of 37° C. Immediately after extraction the Fallopian tube is washed out from the distal (fimbrial) end towards the uterine part with 1 cc of Ringer's solution. The fraction is collected in a watch glass and is subsequently placed in a constant temperature of 37° C. Each Fallopian tube is washed out at least twice, and between the washings the tube is squeezed gently with a pair of tweezers. If the second fraction contains ova, a third washing is made.

The ova are counted under a dissection microscope (40 ×), and their number is compared with the number of ruptured follicles observed. On some occasions there are more ova than apparent follicle ruptures.

The alteration in technique after the first experimental year, with regard to the time when the ova are collected, is due to the fact that the ova have not divided eighteen or nineteen hours after mating. The first division takes place after twenty-one to twenty-four hours. If a division or the commencement of a division is taken as proof that the ovum is fertile, it is possible to separate normally fertilized ova from abnormal ova. Throughout the experimental period only very few cases of abnormal ova have been observed. There have been instances in which the *zona pellucida* has burst and the cytoplasm has evidently invaded the tubal fluid or the solution used for washing. The washing itself does not seem to have any directly harmful effect on the ova. These abnormal ova have been left out of consideration, whereas all other ova have been transplanted.

VOL.
31
1950

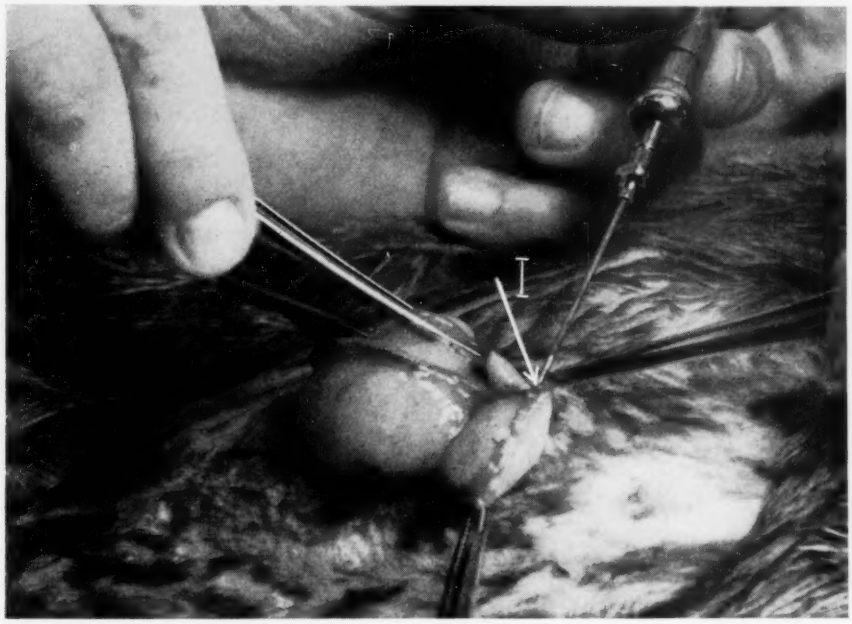

Fig. 4. Transplantation of ova. The cannula containing the ova is directed to the aperture of the Fallopian tube (shown by arrow I). (Photo. I. DYRENDAHL.)

The receptor is anaesthetized and placed on its back on the operating table. The anaestetic used has been Numal-Roche (di-ethylamine salt of allylisopropyl-barbituric acid in a 10 % glycerine-alcoholic aqueous solution). The injection is given intravenously in a dose of 1/2 cc per kgm. body weight and soon causes a deep and rather protacted anaesthesia followed by a sleep period of four or five hours. This is probably of great importance, as the risk of the ova being carried out into the abdominal cavity by peristaltic tubal movements is considered to be materially reduced. In only one instance has extra-uterine pregnancy been observed after transplantation of ova (see below). In this case the ova have probably been washed out into the abdomen after the artificial implantation in the Fallopian tube. After the anaesthetic has become operative, the abdomen is opened by a median incision, approximately three cm long, through the *linea alba*, which ensures that no loss of blood occurs. The incision is made seven or eight cm below the *sternum*. It is then not difficult to extract the Fallopian tube and follow it, until it is possible to get a firm hold with the tweezers of the *mesosalpinx* under the detached ovary. As a rule, the first coil of the Fallopian tube is directed forward and is only loosely connected with the ovary by means of fimbria. This allows for great mobility, and preliminary

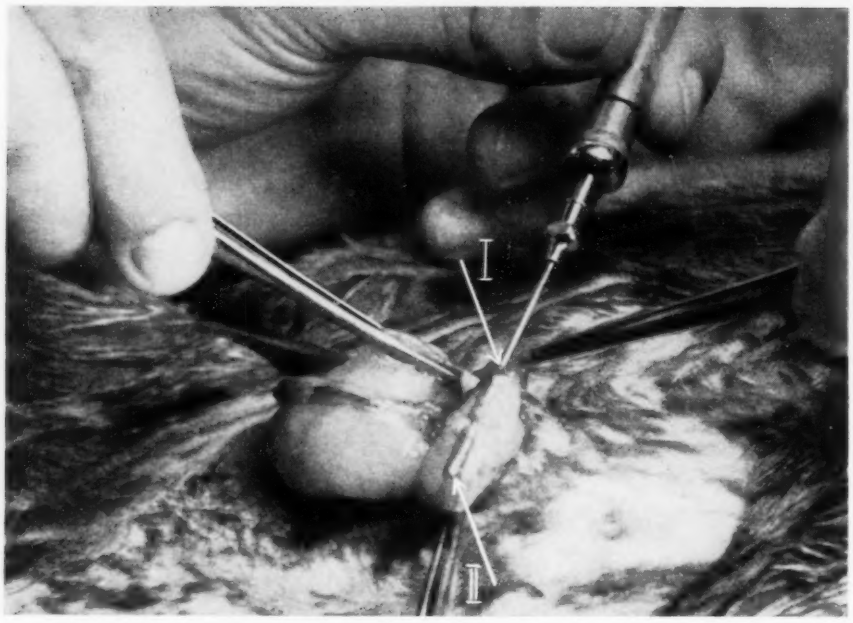

Fig. 5. Transplantation of ova. The cannula is inserted two to three cm into the Fallopian tube. (Arrow II shows the end of the cannula visible through the wall of the tube.) (Photo. I. DYRENDAHL.)

VOL.
31
1950

to the injection of the ova the fimbria is turned forward and obliquely towards the off side, through which the aperture is exposed (Fig. 4). It should be mentioned that CHANG (1948) made the incision through the dorsolateral surface of the trunk.

The injection of the ova is made by means of a special syringe, six cm long and with a lumen of 1.2 mm. In order to prevent the ova from touching metal, the syringe is covered with a paraffin film both on the outside and the inside. The syringe is inserted two or three cm into the tube (Fig. 5). By retracting the syringe very gently during the injection of the ova an even distribution of them is achieved, and it is easier to prevent any fluid from forcing a passage along the sides of the syringe and escaping into the abdomen. It is of major importance that the transplantation takes place with the use of a minimum of fluid, a quantity of about 0.05 to 0.1 cc generally being considered sufficient.

After the ova have been implanted in the Fallopian tube the ovarian ligament (*Lig. ovarii proprium*) is released, and the organs slip into their proper position in the abdomen again. The aperture in the abdominal wall is closed by means of a few sutures which are removed after a couple of weeks.

Various objections may be made against the technique employed in the experi-

ments. One is that the donor is killed, and another that the procedure advocated by PINCUS (1939, 1940) and CHANG (1948) of injecting gonadotrophic hormones in order to induce superovulation has not been used. By using the latter technique the number of donors could be greatly reduced. But it must be pointed out in this connection that the whole planning of the present experiments is against the employment of this technique. For the purpose of the investigations into the post-natal influence, skeletal parts are used to provide material for various measurements, and it is therefore important to have as large a number of individuals as possible in the P-generation in order to be able to assess the variation in the individual measurements with accuracy. The collection of the ova from a living animal is technically somewhat more difficult but no obstacle, however, and it would offer the advantage that the genetic variation could more easily be ascertained. On the other hand, such a procedure will yield a considerable surplus of individuals in the F-generations, just as superovulation will, and would result in a very unequal distribution of the number of individuals in the two generations to be compared. Further, it may be added that in the Spring of 1949 some experiments have been made with injections of gonadotrophic hypophysis hormone (Prep. Ambinon) and fairly good results achieved. Two experiments with combined injections of serum-gonadotrophin (Prep. Gestyl) and chorion-gonadotrophin (Prep. Pregnyl), on the other hand, resulted in the formation of an abnormally large number of cysts in the ovaries, but failed to induce ovulation. A similar observation was made by PINCUS (1939).

PREGNANCY RESULTS OF THE TRANSPLANTATIONS OF FERTILIZED OVA.

The results obtained in the individual years of the experimental period are shown in Table 2. From this it is seen that between February 1946 and May 1949 a total of 249 operations have been performed in connection with the transplantation experiments. Of the 256 animals operated on, 7 (2.75 %) died within a fortnight after the operation. In the course of the experimental period a material improvement in the percentage of pregnant does has been achieved. In the first year, in which the experimental period lasted from 15. Febr. to 3. Sept., 44.6 per cent of the does became pregnant after successful transplantations of ova, in 1947, between 9. Jan. and 7. Oct., the figure was 53.9 per cent, in the third experimental year, from 25. Febr. to 22. Sept., the does became pregnant in 71.6 per cent of the cases, in the last experimental year, from 31. Mar. to 20. May, the percentage decreased to 53.3. The mean pregnancy percentage for the experimental period as a whole is 57.0. This figure

TABLE 2. Summary of transplantations, carried out during the years 1946—1949.

Year	Number of transplantations	Number of litters	Percentage of pregnant does	Number of ova transplanted	Number of young born	Young, in per cent of total number of ova	Young, in per cent of ova transpl. to does with subsequent litters
1946	56	25	44.64	422	79	18.72	41.36
1947	89	48	53.93	508	143	28.15	50.35
1948	74	53	71.62	436	158	36.24	48.77
1949	30	16	53.33	112	35	31.25	55.56
Total	249	142	57.03	1478	415	28.08	48.14

is to be compared with the pregnancy percentage for normally mated does, which in the case of Polish is 41.3 and in the case of Large race 35.6. Considerably better results were obtained by CHANG (1948), who after transplantation of fertilized ova that had been stored for one or two hours at a temperature of 30° C to twenty-four does, ascertained pregnancy in twenty-one, and 54 per cent of the transplanted ova developed into normal young. By storing the ova at 38°C (the same temperature as in these experiments) and not transplanting them until twenty-four hours had passed, he obtained a pregnancy percentage of 61.5, and the number of young amounted to 20 per cent of the transplanted ova. By way of comment on these results it can be said that during some periods in these present experiments a pregnancy percentage of between 90 and 100 has been reached, and whereas CHANG's results are based on a comparatively small number of animals, the present experiments cover a very large number and a very long breeding season. Further, in CHANG's experiments the ovulation is induced by the use of gonadotrophic hormones whereas use has been made here of vasectomized bucks. Most probably the physiological processes necessary for implantation of the blastocysts are induced with greater certainty when CHANG's technique is used, but it is more reasonable to compare these present figures with those resulting from normal matings. The normal figures of 41.3 per cent in the case of Polish and 35.6 per cent in the case of Large race are the averages for the two races for the same number of years, but they do not relate to does in the same sexual condition as the does used as receptors. The majority of the normally mated are does mated for the first time. The number of normal matings of older does has been very small in these experiments, and it is well known that the pregnancy percentage is considerably lower for young than for older does that have been mated before. It was shown by MANRESA (1933) that the average age at the first mating is 6.5 months, whereas the average age at the first fertile mating is 7.5 months.

VOL. 31 1950

The far more favourable results obtained in 1948 are explained partly by minor technical improvements and partly by another factor. The results for the different races are entered in Table 3, and it is clearly seen that by far the most favourable results are achieved when crossbreds act as receptors. The pregnancy percentage for all crossbred does is 69.1 against 53.6 for the pure races. The majority of the transplantations to crossbreds have been performed in 1948/49. It therefore appears that there is a difference between the pure breeds and the crossbreds with regard to the reproductive functions in so far as the implantation of the blastocysts is concerned, that is, a difference in the intensity and the certainty with which the part of the sexual cycle that conditions the implantation is initiated. As a result, pregnancy is more easily induced in the crossbred does. Whether in reality it is a question of fetus mortality with subsequent resorption or an interaction between the technical and physiological factors mentioned is impossible to decide on the basis of this material. The improvement in the results of 1947 over 1946 is no doubt exclusively due to purely technical alterations. It appears from the last column of the table that there is evidently no difference in the power of resistance of the ova to the storing *in vitro*. There is no difference between the pure races and the crosses, either as receptors or donors, with regard to the percentage of the ova that develop into young. The average for the material in its entirety is 48.1. Finally, attention is drawn to the possibility that the difference between the pure races and the crosses as receptors may be of a purely biochemical nature. If that is the case, the more favourable results with the crossbreds would be due to the fact that serologically these represent a mean between the pure races. Certainly

TABLE 3. Summary of transplantations, within the different breeds.

Breed of		Number of animals	Number of litters	Percentage of pregnant does	Number of ova transplanted	Number of young born	Young, in per cent of total number of ova	Young, in per cent of ova transpl. to does with subsequent litters
transplanted eggs	receptor							
Large race	Polish	63	31	49.21	387	91	23.51	48.92
Polish	Large race	42	20	47.62	318	67	21.07	45.27
SL-crossbreds	Polish	43	27	62.79	227	73	32.16	46.50
LS-crossbreds	Large race	38	23	60.53	262	79	30.15	45.93
Pure breeds	Crossbreds	41	28	68.29	210	79	37.62	51.97
Crossbreds	Crossbreds	14	10	71.43	56	21	37.50	52.50
LS-crossbreds	Polish	8	3	37.50	18	5	27.78	71.43
Total		249	142	57.03	1478	415	28.08	48.14

a formation of antigens in consequence of the implantation of alien fetuses does not seem to take place. If that were the case it would be more difficult to use a doe as receptor two or more times, but in the very few instances when it has been done the percentage of pregnant does is not smaller than in the case of those used only once. In support of this view it can be stated that out of nineteen does that were used twice as receptors six did not become pregnant, six became pregnant once, and seven became pregnant twice. Out of eleven does which had had litters after the first transplantation, seven bore litters after the second operation.

OBSERVATIONS ON THE PHYSIOLOGY OF REPRODUCTION.

a. OVULATION.

As stated above, ovulation in the rabbit is brought about by copulation, but it may also be induced through the influence of various stimuli. Generally, there is no difficulty involved in having the animals copulate within the breeding season proper, but from the end of September until the latter half of February, most does (in these latitudes) are in an anoestrous-like state, and a mating is rather difficult to bring about. Even outside this period of quiescence, however, there will be periods within the breeding season proper when a mating certainly takes place, although no ovulation results. This may be explained by the influence of various factors, for example, temperature, changes in the feeding, or the fact that the doe is shedding her coat. Diseases have a similar effect. However, difficulties in that respect have not generally been experienced, and in the cases where the doe has shown signs of reluctance but a mating has nevertheless taken place, the animals experimented on have, before being killed, been subjected to a laparotomic examination for the purpose of ascertaining whether ovulation has actually occurred. There are, however, cases in which no ova have been found, and for which no plausible explanation can be given. A rough estimate has been made to separate the various causes of non-occurrence of ovulation. For 270 does, the cause of failure of ovulation must lie in violent changes of temperature in five per cent of the cases, in six per cent of the cases the cause must be abnormal ovaries, coalescence of peritoneum and fimbria, or a closed tube overcharged with fluid. A further five per cent of the animals were diseased and it was decided to kill them. Before destroying them, however, a mating was attempted, but none ovulated. Barely five per cent of the 270 does showed, for some unknown reason, no signs of ovulation.

For all normally ovulating animals, records have been made of the number of ova found at the washing of the Fallopian tubes. Therefore it is possible to compute with great certainty not only the total number of ova but also any difference between the number released from the left or the right ovary. The

VOL.
31
1950

TABLE 4. Average number of ova produced by the pure breeds.

Breed	Number of animals	Mean number of ova	Standard deviation	Coefficient of variation, %
Polish	133			
left ovary		2.73	1.60	58.46
right ovary . . .		2.79	1.59	56.85
per animal . . .		5.52	1.59	28.78
Large race	71			
left ovary		5.31	2.17	40.85
right ovary . . .		5.42	2.44	44.94
per animal . . .		10.73	2.72	25.37

results are shown in Table 4. For Polish the average is 5.52 ± 0.138 ova per indivudal, and for Large race 10.73 ± 0.323. In spite of less standard deviation in the case of Polish than for Large race, the former is still slightly more heterogenous with regard to the number of ova produced, the coefficient of variation for Polish being 28.78 per cent against 25.37 per cent for Large race. Further, it appears from Table 4 that within either of the breeds there is no difference between the reproductive capacity of the right and the left ovary, a finding which is entirely in accordance with observations made by HAMMOND and MARSHALL (1925) and GREGORY (1932). However, with regard to the absolute and the relative fecundity, there is some discrepancy between the two sets of data. GREGORY states the average number of ruptured follicles to be 3.97 in the case of Polish and 12.88 in the case of Large race. The relative potential "fecundity" is given as 1 ovum per 380 gms. of body weight for Polish, whereas these observations show 1 ovum per 297 gms. of body weight. For Large race the corresponding figures are 434 gms. according to GREGORY's data, against 394 gms. in this material, or the same figure as GREGORY states for Polish. The actual fertility, computed by dividing the average body weight by the average litter size is found to be 368 gms. per young in the case of Polish and 508 gms. per young for Large race. GREGORY's results are 471 gms. and 567 gms. respectively. The difference between the potential and the actual fertility would then be explained either by the fact that not all ova become fertilized or by uterine death of the embryos (provided of course that the material can be considered fully representative of the two breeds). This gives a "viability" of the ova of 80.8 per cent in Polish and 77.6 per cent in Large race. These figures are the same as those given by GREGORY.

It is generally supposed that the body weight is of decisive importance for

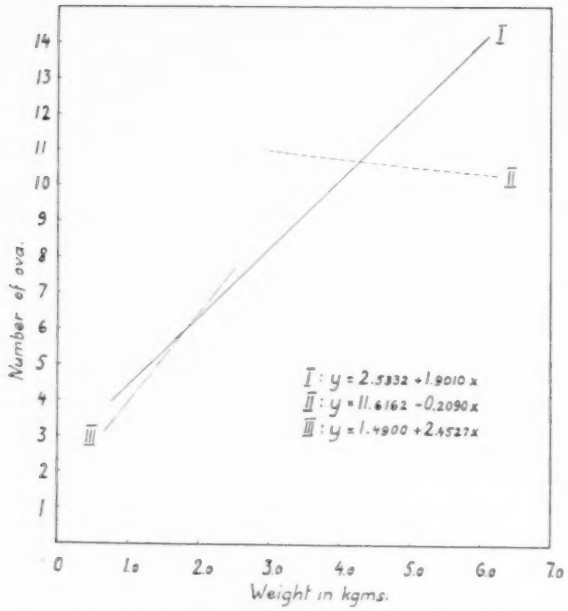

Fig. 6. Regression lines for produced ova on weight of doe. *I* is the total regression; *II* is the line for Large race; *III* is the line for Polish. $r_{II} = 0.0309$ ($P > 0.2$). $r_{III} = 0.4130$ ($P < 0.001$).

the potential (as well as the actual) fertility of the animal in so far as the difference between breeds is concerned. GREGORY (1932) ascertained an increase in the body weight of slightly less than 460 gms. per unit increase of ruptured follicles, i.e. a regression of ruptured follicles on body weight of 2.18. However, within the different breeds there seems to be considerable divergence, as is seen

TABLE 5. Analysis of regression of number of ova on weight of doe.

Source of variation	Degrees of freedom, D.F.	Remainder sum of squares	Remainder mean square	Variance ratio
Total	202	884.20		
Difference between intercepts on the y-axis .	1	46.73	46.73	11.21[3]
Remainder	201	837.47	4.17	
Difference between slopes of regression lines . .	1	43.01	43.01	10.83[2]
Error	200	794.46	3.97	

[2] $P < 0.01$; [3] $P < 0.001$.

VOL. 31 1950

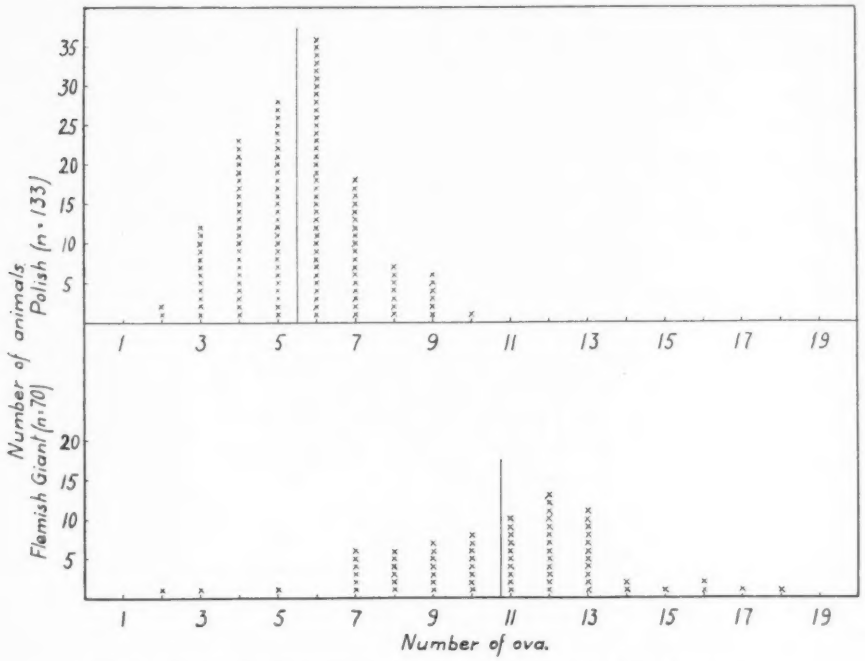

Fig. 7. Variation in the number of ova produced by the two pure breeds.

from Fig. 6, in which the regression lines of the individual races in relation to the mean regression line are shown. From the coefficients of regression it appears that there is a steeper line for the Polish breed than for the total material, and further that the regression within Large race is, if anything, negative (but not significantly different from zero). The difference between the lines for the two breeds is highly significant ($P < 0.001$) (Table 5). Without entering into a detailed discussion of the results the probability is suggested that the relationship between body size and potential fertility follows a curvilinear regression, in so far as ovulation after natural mating is concerned.

The largest number of ova found after a normal mating was in a Vienna Blue doe, which 21 1/2 hours after the mating had ten ova in her left and eight in her right Fallopian tube, a total of eighteen ova in all. The largest number found in a Polish doe was ten, nine of which were found in the left and one in the right tube, 25 hours after the mating. Fig. 7 shows for each of the races the distribution of the total number of ova found in each individual doe.

b. DIVISION OF THE OVA.

The fertilization of the ova takes place between 11 1/2 and 14 hours after the mating (PINCUS 1930, PINCUS and ENZMANN 1932, HAMMOND 1934), but not

until eight to nine hours later does the first division of the ovum start, and twenty-five hours after the mating all the ova are at the two-cell stage. GREGORY (1930) states that the division takes place 22 1/2 hours ± 1 hour after the mating, adding that ova at the two-cell stage may be found from 21 1/2 hours until 31 1/4 hours, but most of them seem to enter the four-cell stage after about 25 1/2 hours after the mating. In the present investigations the average for ova at the two-cell stage has been 24 hours 0.8 minutes ± 2.4 minutes in the case of Polish with a standard deviation of 55.4 minutes. For Large race the corresponding figures are 24 hours 2.5 minutes ± 2.4 minutes with a standard deviation of 43.3 minutes. The very slight difference between the races is clearly not significant. As late as 25 hours after the mating apparently fertilized ova in the one-cell stage have been observed in both races, whereas the first ova in the two-cell stage have been observed after 20 hours 45 minutes in the case of Polish and 22 hours 30 minutes in Large race. Ova at the four-cell stage have been found in the Polish breed as early as 22 hours 30 minutes and in Large race one half hour later. These data have been entered in Table 6. The curves in Fig. 8 are based on the mean development stage of the eggs, calculated according to the formula $\frac{1n_1 + 2n_2 + 3n_3}{n_1 + n_2 + n_3}$, where 1, 2 and 3 represent the cell-stage one, two and four resp. and n is the number of eggs. The difference between the two races is statistically significant ($P < 0.05$). As would be expected, the division of the ova takes place in the rabbit considerably earlier than in larger animals, ova in the two-cell stage having been found in the sheep 39 hours after mating (CLARK 1934, GREEN and WINTERS 1945), and after 48 to 50 hours in the cow (HARTMAN *et al.* 1931, WINTERS *et al.* 1942).

During the passage of the ova through the tube, the zona pellucida will be covered by a thick albuminous layer (GREGORY 1930, PINCUS 1930). Frequently very great differences in the thickness of the layer will be observed

TABLE 6. Age of ova at varying stages of development.

Stage of development	Polish		Large race	
	hours	minutes	hours	minutes
One-cell, last observed	25	00	25	00
Two-cell, first observed	20	45	22	30
Two-cell, last observed	26	30	25	30
Four-cell, first observed	22	30	23	00
Average age at two-cell stage	24	0.8	24	2.48
	$SE_M = \pm 2.4$ min.		$SE_M = \pm 2.4$ min.	
	SD = 55.4 min.		SD = 43.3 min.	

VOL. 31 1950

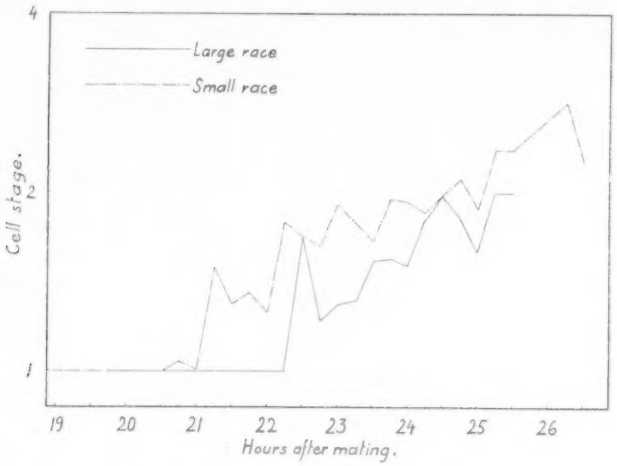

Fig. 8. Development stage of ova for the two pure breeds.

in ova from different donors. In order to ascertain any possible interdependence between the thickness of the albuminous layer and the number of developed fetuses a point scale has for the majority of the ova been used to express the thickness of this albuminous layer. The albuminous layer presumably has a protective effect on the ovum, and if this is the case, a larger number of fetuses after transplanted ova with a strongly developed albuminous layer than after ova with no layer or an insufficiently developed one may be expected. The results are shown in the computation below, in which the basis of distribution has been the donor, irrespective of whether the ova are "pure breed" or "crossbred".

Albuminous layer:

Large race, 594 ova in all	thin or no layer	medium	thick
pct. transplanted ova	44.78	33.67	21.55
pct. of the young developed	36.36	46.02	17.62
Polish, 565 ova in all			
pct. transplanted ova	16.99	31.15	51.86
pct. of the young developed	18.52	25.31	56.17

A χ^2-analysis shows that in Large race there is a difference in the percentage of fetuses between the three classes, ova with a medium albuminous layer giving a larger number of fetuses ($P < 0.01$), but otherwise the figures do not show any definite trend. There is the same percentage of fetuses developed from the ova in which no visible quantities of "albumin" have been deposited or that

have had a poorly developed albuminous layer as from the ova that have had an ample albuminous layer. Consequently it is more probable that the development of the albuminous layer is exclusively, or at least for the greater part, dependent on the time, for in the Polish breed considerably more ova at the two-cell stage are found at the time of examination. By comparing the figures for the development stage of the ova, as shown below, with the percentages in the above table, it is seen that for Large race there are 44.78 per cent ova with a deficient albuminous layer or none at all, against 52.02 per cent ova on the one-cell stage. For Polish there are 16.99 per cent with a deficiently developed albuminous layer, which corresponds to 16.10 per cent of the ova being at the one-cell stage.

The development stage of the ova:

Large race	one-cell stage	two-cell stage	four-cell stage or above
pct. ova	52.02	47.14	0.84
Polish			
pct. ova	16.10	77.88	6.02

The deposition of the albuminous layer must be supposed to be largely dependent on the physiological condition of the mother. Whether the layer exerts a protective influence on the ova during the storage *in vitro* it has not been possible to ascertain with any certainty.

c. IMPLANTATION AND PREGNANCY.

There is, as just mentioned, no correlation established between the thickness of the albuminous layer and the number of fetuses born. Therefore it must be regarded as most probable that the physiological condition of the Fallopian tubes is not of any great importance for the implantation of the blastocysts. When it is a matter of normally fertilized ova the implantation is exclusively dependent on the physiological condition of the uterine wall. First and foremost the formation of corpora lutea play a decisive part. These are problems which will not be discussed in detail here, but suffice it to say that it seems improbable that the method of mating to vasectomized bucks for the purpose of inducing ovulation should have any restrictive effect on the preparation of the mucous membranes in the uterus for the reception of the blastocysts. A restrictive factor might rather be found in serological differences between the two races used, but as stated before, no formation of antigens seems to occur. Whether during the pregnancy a resorption of already implanted fetuses has taken place has not been examined. However, it is a fact that such resorption happened during the snuffle epizootic in the Spring of 1949. In one case the receptor

VOL. 31 1950

died twenty-three days after the transplantation of ova had taken place, and in an autopsy, four macerated fetuses were found, two in each uterine horn. It was impossible to separate placenta from the fetuses. The appearance of the highly resorbed fetal remains indicated that the fetuses had died some days earlier. This occurrence will be discussed further on page 93. During the same period a couple of operated does were observed which began nesting thirty to thirty-one days after the operation, but no fetuses or fetal remains were found, and an autopsy performed a couple of days later showed that the uterus had an entirely normal appearance. In these cases it is no doubt a question of resorption of the fetuses, for when it is only a matter of pseudo-pregnancy the does begin plucking themselves and nesting about sixteen to seventeen days after the mating, at which time the involution of corpora lutea has begun.

Only in one case has a miscarriage been observed. A cross fetus between Large race ♂ and Polish ♀ was aborted twenty-two days after the transplantation of seven ova to a Large race receptor. Including placenta, the fetus weighed 6 gms., which indicates that until the abortion the development had been normal. HAMMOND (1935) states, that the weight of a twenty days old fetus plus fetal membranes is 5.7 gms. in small litters for a breed of rabbits that had an adult weight of 3.5 kgms. This corresponds approximately to the weight of the receptor in the case mentioned.

As previously shown, about 50 per cent of the transplanted ova have developed into young in these transplantation experiments. This is somewhat less than stated by HAMMOND (1921), who found that approximately 70 per cent of the ova developed into normal fetuses, almost 10 per cent were identified as atrophied fetuses, and a little more than 20 per cent of the ova did not become fertilized or the fetuses atrophied at a very early stage. Computed on the basis of the observed number of ova produced by the individual does and the average size of the litter, the normal percentage should range around 80 in the case of Polish, and slightly below that figure in the case of Large race (see page 33). In the following the results from the various experimental groups with regard to the implantation result, expressed in percentage of young out of transplanted ova, will be discussed. As has previously been briefly mentioned, no direct investigations into this problem have been made, for which reason it is not possible to state definitely whether implantation has actually taken place of a greater number of blastocysts than indicated by the number of young born in the individual litters.

*A*II. *Large race borne by Polish does.*

Several attempts have been made to obtain litter sizes in Large race borne by Polish, which should correspond more approximately to the normal litter size in Large race than to the average litter size in Polish, but in no case has

a larger litter size than six been obtained (Table 7). In one instance ten fertilized ova have been transplanted without any litter having been obtained, in four cases nine fertilized ova have been transplanted, and after these transplantations pregnancy has been observed in three cases, only four young, however, being born, that is 11.11 per cent of the transplanted ova. Evidently the most favourable results are achieved by the transplantation of five ova, when 50 per cent of the does have become pregnant, and 42.5 per cent of the transplanted ova have developed into young. On the basis of the limited number of individuals in each group it is difficult to draw any definite conclusions as to which is the optimum number of ova to transplant. A χ^2-analysis shows a significant difference ($P < 0.01$) between the various classes, but it is not possible to distinguish between the many interacting factors that may have an adverse effect on the fate of the ova. The possibility cannot be ignored that perhaps in those cases where many ova have been transplanted to a receptor the ova may not have been fertile or there may have been a large percentage of infertile ova. This, however, appears to be rather improbable, since the ova of any one donor are as a rule distributed between two or more receptors. Likewise it might be presumed that the transplantation of a number of ova in excess of the normal number of ova released for the race has considerably reduced implantation possibilities. This could mean either that there is a purely quantitative connection between the number of blastocysts that can be implanted and the number of corpora lutea formed in the ovaries of the receptor after the ovulation, due to the available quantity of progesterone. Alternatively, it may be that the implantation percentage is the same whether many or few ova are transplanted, and therefore greater uterine mortality will occur when many eggs are transplanted. This problem can be examined by always transplanting a number of ova that is proportional to the number of ruptured follicles, or by unilateral ovariectomy and transplantation of a number of ova constant for the race. The discrepancy between the numbers of transplantations shown in Tables 3 and 7 is due to the fact that the first list includes three transplantations to Polish where the receptor has been mated to fertile buck instead of to vasectomized buck. Therefore normal Polish as well as Large race were born in the litters in question. In addition, the first table includes four transplantations where the fetuses have not been of pure Large race extraction.

*B*II. *Polish borne by Large race does.*

In this experimental group a pregnancy percentage of 46.34 has been recorded, and 45.07 per cent of the ova that have been transplanted to pregnant does have developed into young. Only 20.51 per cent of the total number of transplanted ova have developed into young (Table 7).—The difference between these figures and those in Table 3 is once more due to the fact that ova were

VOL.
31
1950

TABLE 7. Development of ova into young and percentage pregnant does within "litter" sizes.

Number of ova transpl.	Polish as receptor for Large race			Large race as receptor for Polish			Polish as receptor for SL-crossbreds			Large race as receptor for LS-crossbreds			Crossbreds as receptor for pure breeds		
	n	Young, %	Pregnant does, %	n	Young, %	Pregnant does, %	n	Young, %	Pregnant does, %	n	Young, %	Pregnant does, %	n	Young, %	Pregnant does, %
2							2	0	0				4	12.5	25.0
3	1	33.3	100.0	1	0	0	2	16.7	50.0	3	33.3	33.3	4	33.3	50.0
4	8	15.6	37.5	6	30.0	40.0	8	18.8	25.0	3	25.0	33.3	4	37.5	50.0
5	8	42.5	50.0	2	20.0	50.0	14	35.7	78.6	4	30.0	50.0	6	46.7	83.3
6	19	29.8	47.4	8	33.3	62.5	12	23.6	66.7	5	20.0	40.0	20	38.3	80.0
7	12	23.8	50.0	5	14.3	40.0	3	52.4	100.0	10	41.4	80.0	1	28.6	100.0
8	3	0	0	6	50.0	80.0	—	—	—	5	22.5	60.0	1	75.0	100.0
9	4	11.1	75.0	2	5.6	50.0	1	77.8	100.0	4	16.7	50.0	1	0	0
10	1	0	0	5	4.0	20.0	—	—	—	2	60.0	100.0			
11				2	36.4	50.0	—	—	—	1	9.1	100.0			
12				1	0	0	—	—	—	—	—	—			
13				1	0	0	1	46.2	100.0	—	—	—			
14				1	0	0				1	28.6	100.0			
15				1	13.3	100.0									
	56	23.6	46.4	41	20.5	46.3	43	32.2	62.8	38	30.2	60.5	41	37.6	68.3

n = number of does.

transplanted to a doe that had been mated to a fertile buck, and both pure Polish and normal Large race were born in the litter.

In several cases the number of ova transplanted is a little below or a little above the average number of ova released in Large race. Eleven ova were transplanted to each of two does, and a very favourable result was achieved, for one became pregnant and bore eight living young. Apart from this litter, however, pregnancy after transplantation of a large number of ova has been observed in only one case, involving a doe which, after having received fifteen ova, bore only two young. In no instance have twelve, thirteen, or fourteen transplanted ova resulted in young being born, but it would be hazardous to draw any definite conclusions on the basis of these figures as in each of the cases only one doe has been used. The largest number of young is to be found in the group to which eight ova have been transplanted. In this group 45.83 per cent of the ova have developed, and it is this favourable result which influence the χ^2-test and results in significant differences between the various classes. Nevertheless, there is nothing to indicate that these results are at variance with the suggestion made in a previous section that a quantitative correlation exists between the implantation of blastocysts and the formation of corpora lutea.

VOL
31
195

*C*11. *F_1-crossbreds from Polish ♂ and Large race ♀, borne by Polish does.*

In this experimental group the pregnancy percentage is somewhat higher than for the two groups previously discussed. A total of 227 ova have been transplanted to 43 mothers, and of these 27, i.e. 62.79 per cent, have yielded litters (Table 7). In contrast to what was found in the case of Large race borne by Polish, litters have in this instance been obtained after transplantation of a larger number of ova. These improved results can in all probability be attributed largely to more experience and to improved technique of transplantation. Most of these transplantations have been made in the second experimental year when as a rule the collection of the ova took place at a little later stage than in the first year. In one instance a litter of seven was born after transplantation of nine ova, and in another six young were born after transplantation of thirteen ova. On the other hand no litters resulted after the transplantation of only two ova, which was performed in but two cases, and after transplantation of three and four ova a development percentage of only 16.67 and 18.75 respectively has been ascertained. The best results have been obtained in the groups to which seven, nine, and thirteen ova have been transplanted. A χ^2-test shows that there is a small, but significant difference between the classes, mainly due to the very favourable result after transplantation of nine ova ($P < 0.05$).

D_{II}. F_1-crossbreds from Large race ♂ and Polish ♀, borne by Large race does.

In this group too a satisfactory pregnancy percentage has been obtained in comparison with the pure races. Of 38 receptors 23 have become pregnant, i.e. a pregnancy rate of 60.53 per cent (Table 7). A total of 172 ova have been transplanted to the "pregnant" does, and an aggregate of 79 young gives a development percentage of 45.93, which is very much the same as in the other groups. Transplantations of ten ova have yielded the best results. It has taken place in two cases, one doe bearing a litter of five and the other a litter of seven young, or in other words an average of 60 per cent of the transplanted ova developed. Apart from this case there is no great difference in the development percentage between the individual classes, and a χ^2-analysis does not show any significant difference either.

VOL. 31 1950

E. Pure breeds born by crossbred does.

By far the best result has been achieved by using crossbred does as receptors for the pure breeds. In all, 40 transplantations have been performed, and the average for 1948 and 1949 has been pregnancy in 68 per cent of the cases (Table 7). The average development percentage too is a little higher, 37.62 per cent of all transplanted ova having developed into young. The development percentage in the case of does becoming pregnant after the transplantation is 51.97. The largest number of ova transplanted within this group is nine, but it did not result in any young being born. In one case eight ova were transplanted, when six young were born, and in another, seven ova were transplanted and two young were born. If the individual cases mentioned are left out of consideration, the highest pregnancy percentage has been reached in the groups in which five and six ova respectively have been transplanted. Here the pregnancy percentage has been 83.33 and 80.00. The development percentage has ranged somewhat above the average, but a χ^2-test shows no difference between the classes.

The small group of crossbreds borne by crossbreds will not be dealt with in detail, but it can be mentioned that the pregnancy percentage is 71.43, the aggregate development percentage is 37.50, and in the pregnant does 52.50 per cent of the ova have developed.

d. SEX RATIO.

Frequent attempts have been made to alter artificially the ratio between the two sexes in various species of animals. The methods are based on changing the pH in the vagina or they are attempts to bring about an alteration of the sex ratio by influencing the spermatozoa so that fertilization of the ova by either androsperms or gynosperms is favoured.

In experiments with transplantations of fertilized ova on the other hand, it is conceivable that differences in the power of resistance of the male or female blastocysts to manipulation might affect the relative numbers of male and female individuals born. From the present data for 654 normally born young, the sex ratio is 52.75 per cent and for 368 transplanted the sex ratio has been 47.01 per cent. All litters for which details of the sex of one or more young are not available have been left out of account. A slightly larger number of males have been found in Large race than in Polish, but the greatest deviation has been observed in the LS-crossbred group. However, the number is small, and no significant difference from the normal can be shown. The sex ratios for the different groups have been entered in Table 8, and it is seen that the sex ratio for Polish borne by Large race is very slightly above the average sex ratio for the normal individuals. In all the other groups of transplanted young the percentage of males is lower than in the normal group and for both Large race borne by Polish and crossbred between Large race male and Polish doe borne by Large race doe, very much below the expected percentage. The difference between the normal and the transplanted individuals is not significant ($P < 0.1$), and therefore any harmful effect which might result from the manipulation of the ova *in vitro* can be disregarded.

The small deviations found in a few of the groups are probably purely accidental. It is very difficult to find any plausible explanation why two such different groups as Large race borne by Polish or LS-crossbreds borne by Large race (sex ratio 41.03 %) should react differently to the storage *in vitro* as zygotes, or show greater fetal mortality for males *in utero*, than all the other groups (sex ratio 48.62 %, $P < 0.3$). It is generally supposed that there

TABLE 8. Sex ratio.

Group	Number of animals	Males, %	Females, %
A_I. Normal Large race	198	53.03	46.97
B_I. Normal Polish	331	50.45	49.55
A_{II}. Large race, transpl.	78	41.03	58.97
B_{II}. Polish, transpl.	64	56.25	43.75
C_I. Normal SL-crossbreds	45	55.56	44.44
D_I. Normal LS-crossbreds	80	60.00	40.00
C_{II}. SL-crossbreds, transpl.	73	50.68	49.32
D_{II}. LS-crossbreds, transpl.	78	41.03	58.97
E. Pure breeds born by crossbreds	75	48.00	52.00
Total normal	654	52.75	47.25
Total transplanted	368	47.01	52.99

is a considerably greater zygotic and embryonic mortality among males than among females, but whether that is due to the fact that males are heriditarily weaker than females, or to sexlinked semilethal genes, or whether it is a matter of purely physiological differences between the sexes with regard to the rate of metabolism, is still unknown (See: LAWRENCE 1941).

*

In several cases the birth of fetuses of Large race developed in Polish has been difficult (*fetal dystocia*), and it has been necessary to give injections of oxytocin in order to intensify uterine contractions. The fetuses have in these cases been so big that they have not been able to survive the birth, and a post-mortem examination has disclosed cerebral hemorrhage and a dislocation of the organs in abdomen, right down to the pelvis. The fetuses have been removed by hysterotomy in four cases, one of which was a cross fetus developed in a crossbred doe, the three other being Large race fetuses developed in Polish. In the first case the fetus was not removed until thirty six days after the egg transplantation was made. At that time death had occurred, but there were no visible signs of resorption. In the other cases the operation did not take place until after the parturition had started in the natural way and palpation had shown that there were at the most two fetuses in the uterus. The young survived the birth. In one case a dead fetus had to be removed surgically from vagina.

It should be mentioned here that extra-uterine development of two fetuses has been observed in a Polish doe. The case is of particular interest because one fetus had developed normally and was born viable after the normal gestation period, whereas two fetuses were found situated in the abdomen with the fetal membranes developed in *peritoneum viscerale*. It does not seem to be a case of a diverticle from the uterus but of a purely extra-uterine development of maternal placenta. One fetus was situated somewhat ventrally-laterally of *colon* on the right, while the other was found dorsally of *caecum* with the membranes developed in between the loops of the *ileum* in the left side of the animal. There was no connection with the primary reproductive organs. Doubtless the ova have slipped out into the abdominal cavity after the transplantation.

EXPERIMENTAL RESULTS.

INFLUENCE OF DIFFERENT FACTORS ON THE BIRTH WEIGHT.

Numerous investigations have shown that the size of the fetuses at birth is dependent on a series of factors, such as the season and the length of the gestation period. In the following a survey will be given of the modifying effect of some of these environmental factors on the birth weight.

a. LENGTH OF THE GESTATION PERIOD.

It was shown by KNAPP *et al.* (1941) that in cattle the length of gestation is highly correlated with the weight of the newborn calf, whereas FITCH *et al.* (1924) and PAIM (1944) found that the gestation period had but slight influence on the birth weight. As for multiparous animals, MINOT (1891) showed that in guinea pigs the average birth weight per young is less in large litters than in small ones because the large litters have a shorter gestation period. An increase in the number of young in the litter up to four or five causes a decrease in the gestation period of a couple of days. WRIGHT (1921) found a correlation between the length of gestation and the litter size of —0.4444 and between the length of gestation and the birth weight of 0.5547. IBSEN (1928) did not think that the length of the gestation period had any great influence on the weight but considered the litter size to be a modifying factor of greater importance. EATON (1932) found a correlation between the gestation period and the birth weight of 0.44 (non-inbred material), but showed at the same time that the length of the gestation period was correlated with the litter size. With a constant litter size, the partial correlation was 0.23. For mice, BLUHM (1929) observed that the litter size exerted greater influence on the birth weight than did the length of the gestation period, a finding which is in accordance with the results presented by KOPEĆ (1924). WISHART and HAMMOND (1933) examined the correlation between litter size, birth weight, and length of gestation period for three different strains of rabbits, two of which (C and E) had a weight of about 3.5 kgms., while the third strain (F) had a weight corresponding to that of Polish in these present investigations. They found that in the case of the C strain the birth weight was not affected by the length of the gestation period ($b = -0.67$), but the E and F strains showed an increase of the birth weight from 1 1/2 to 4 gms. with a one-day extension of the gestation period ($b = +2.57$ and $b = +3.18$).

In order to analyse the effect of the gestation period on the birth weight within the various experimental groups as well as within the normal litters a calculation of the correlation between these factors has been made; but if it is assumed *a priori* that the gestation period and the fetal weight have a reciprocal influence, it seems logical to suppose that the total weight of fetuses is thereby affected. The calculations have therefore been carried out as a correlation between the period of gestation and the weight of the litter. Table 9 shows the coefficients of both correlation and regression. The highest correlation exists in the experimental group comprising crossbred offspring borne by Polish does, whereas the lowest is found in normal Polish. Common to all the groups is the negative correlation between the development period *in utero* and the total weight of the young in any one litter. This is in full

TABLE 9. Regressions (b) of litter weight, litter size and weight of mother on length of gestation; and the corresponding coefficients of correlation (r).

Group	Number of litters	Litter weight, gms.		Litter size, number		Weight of mother, kgms.	
		r	b	r	b	r	b
AI Normal Large race	56	−0.3676[2]	−70.86	−0.4727[3]	−1.53	−0.2947[1]	−0.18
BI Normal Polish . .	102	−0.2155[1]	−15.15	−0.3100[2]	−0.63	0.0082	—
AII. Large race, transpl.	25	−0.4200[1]	−26.60	−0.4846[2]	−0.83	0.0805	0.02
BII. Polish, transpl. . .	19	−0.5322[1]	−47.98	−0.5765[1]	−0.93	−0.0704	−0.06
CII. SL-crossbreds, transpl.	27	−0.8071[3]	−65.01	−0.7376[3]	−1.47	−0.2010	−0.06
DII. LS-crossbreds, transpl.	22	−0.4550[1]	−37.31	−0.4213[1]	−0.67	−0.0765	−0.04
E. Pure breeds born by crossbreds	26	−0.3047	−25.23	−0.3650	−0.53	0.4763[1]	0.24

[1] $P < 0.05$; [2] $P < 0.01$; [3] $P < 0.001$.

OL. 31 950

accordance with the results obtained by KOPEĆ (1923), HAMMOND (1934) and others.

The length of the gestation period has also been correlated with the litter size. The coefficients are likewise shown in Table 9, and it will be seen that in this case also normal Polish has the lowest correlation between the gestation period and the litter size, and transplanted SL-crossbreds has the highest correlation. In agreement with the foregoing it is seen that all correlation coefficients are again negative. The regression coefficients indicate that the period of gestation is reduced one day by an increase of two thirds to one and a half of the number of young in the litter. The strongest influence of litter size on the gestation period is found in normal Large race and in transplanted SL-crossbreds. For the two normal groups it appears that the average gestation period is a little shorter for Polish than for Large race does (Table 10), whereas for all experimental groups the gestation period is about one day longer. The difference is probably due rather to a difference in the litter size than to an absolute size difference between the does, and any real racial difference seems to be out of the question.

If the correlation between the size of the doe and the length of the gestation period is calculated, a negative correlation is found in the case of Large race, whereas there is no apparent correlation between these factors in the Polish breed in so far as the normally born young are concerned (Table 9). In the case of does which bear transplanted litters there is a tendency for the larger mothers to have a slightly shorter gestation period, but within this group the litter size plays a very great part in causing a decrease of the gestation period.

TABLE 10. Length of gestation period and corresponding mean litter size, mean litter weight and weight of doe.

	Number of does	Days	Mean litter size, number	Mean litter weight, gms.	Mean weight of the doe, kgms.
A_I. Normal Large race . .	56	31.5	8.13	516.1	4.76
B_I. Normal Polish	102	31.1	4.48	177.1	1.70
A_{II}. Large race, transpl. . .	25	31.6	3.12	170.2	1.49
B_{II}. Polish, transpl.	19	32.5	3.37	195.3	4.17
C_{II}. SL-crossbreds, transpl. .	27	31.8	2.70	147.6	1.58
D_{II}. LS-crossbreds, transpl. .	22	32.4	3.55	224.9	4.04
E. Pure breeds born by crossbreds	26	31.9	2.88	179.9	2.98

The average length of the gestation period within the various groups is shown in Table 10. The shortest gestation period, 31.12 days, is found in normal Polish, which is in agreement with PICKARD (1930) who found the gestation period to be 31.22 days (9 litters) for the same race, but whereas he found the gestation period for Flemish Giant (6 litters) to be 32.17 days, the estimate in these present investigations is 31.45 days. However, it must be emphasized that in this figure are included a few normal litters by Vienna Blue. MENSHOW (1935) stated the length of the gestation period to be 30.79 days in the case of this latter race. ROSAHN *et al.* (1935) found a gestation period of 31.79 days for Flemish Giant with an average litter size of 7.14 and a correlation between gestation period and litter size of — 0.735. For the Polish breed an average gestation period of 30.37 days was found with an average litter size of 3.92. The correlation coefficient was — 0.048.

In these investigations, the shortest period of gestation for the normal groups is 29 days in the case of Polish and 30 days in the case of Large race. This is partially in agreement with observations made by ROSAHN *et al.* (1935), who stated the shortest period for Polish to be 29 1/2 days and for Flemish Giant 31 days. The same authors found the longest period of gestation for Polish breed to be 31 1/2 days and 34 days for Flemish Giant. The corresponding figures in these investigations are 34 days for Polish and 33 days for Large race. The distribution is shown in Fig. 9, from which it will be seen that 50 per cent of the does of both races have a gestation period of 31 days, but while 22.5 per cent of the Polish does have a period of 30 days and the same proportion 32 days, only 11 per cent of the Large race does have 30 days, and 16 per cent have a gestation period of 33 days.

As emphasized above, the size of the litter exerts a certain influence on the length of the gestation period. MANRESA (1933) found a correlation of — 0.26 with a mean litter size of 6.18 and a gestation period of 31.49 days, while

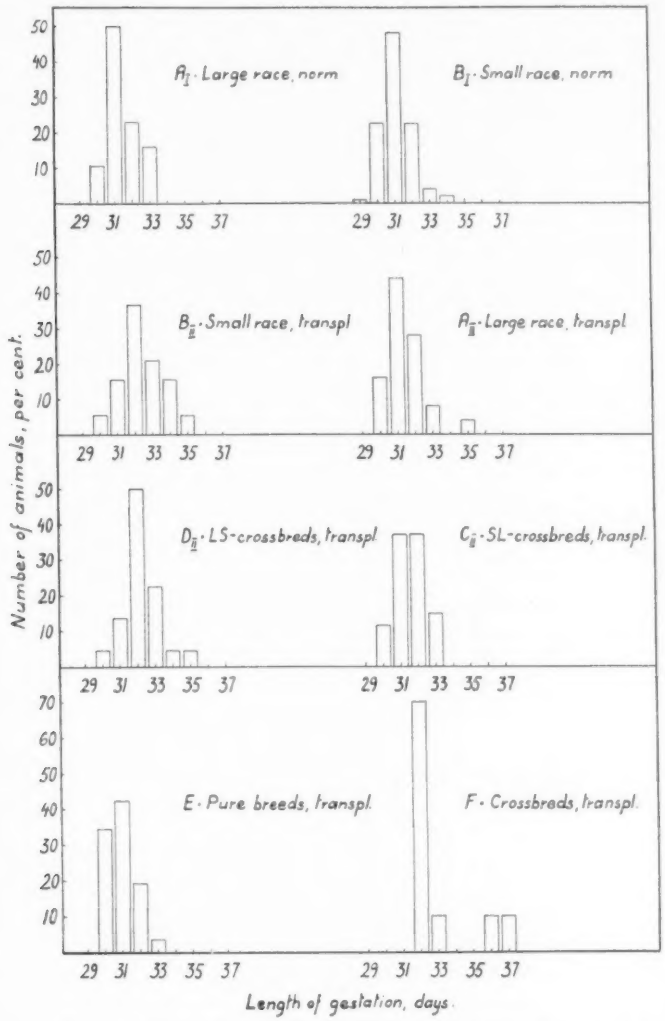
A I · Large race, norm
B I · Small race, norm
B II · Small race, transpl
A II · Large race, transpl
D II · LS-crossbreds, transpl
C II · SL-crossbreds, transpl
E · Pure breeds, transpl
F · Crossbreds, transpl
Number of animals, per cent.
Length of gestation, days

Fig. 9. Length of gestation period for the different groups.

ROSAHN *et al.* (1935) found the average correlation within breeds to be —0.3004. When the material in these investigations is grouped according to maternal breeds, i.e. irrespective of whether they are mothers of normal litters or transplanted litters, an average correlation between the period of gestation and the size of the litter of —0.4941 is found in the case of Polish and —0.6448 in Large race while the correlation between the period of gestation and the weight of the litter is —0.3557 for Polish and —0.6057 for Large race. All four coefficients are highly significant ($P < 0.001$).

b. SEASONAL INFLUENCE.

It is known from the study of other domestic animals that the reproductive functions are to some extent subject to seasonal influence and this also applies to the rabbit. HAMMOND and MARSHALL (1925) pointed out that the breeding season of the rabbit was partially restricted to the months of April to September (in England), while during the Winter months it was more difficult to bring about matings. Very much the same conditions apply to Middle Sweden. PICKARD (1930) arrive at a similar result with regard to the number of litters born, and likewise found an increase in the size of the litters from early in the year until late in August. In the Autumn months he recorded a considerably smaller litter size. MANRESA (1933) examined the birth frequency and ascertained that this was lower at the height of Summer than in the other seasons. The gestation period does not appear to be affected by the season to any noticeable extent (ROSAHN *et al.* 1935). Besides the factors mentioned here it is also quite possible that other factors such as the feeding may have a seasonal-like influence on the sexual functions, and so exert a modifying effect on the birth weight.

In the present investigations the seasonal influence has been examined as far as the normal groups are concerned by dividing up the material into classes according to the birth month of the litters. The results are shown in Table 11, from which it will be seen that in no case has any variation due to the season been ascertained. For the normal group of SL-crossbreds the available material does not permit a division "between months within litter sizes", as in no case were litters of the same size born within the same month. From the table it will be seen that it is not possible in this material to trace any significant seasonal influence on the birth weight, and it is therefore considered valid to leave this factor out of account, both for the normal and the transplanted litters. In all four groups by far the largest part of the variation is to be found

TABLE 11. Seasonal influence on birth weight for normal groups.

Source of variation	Large race		Polish		SL-cross-breds		LS-cross-breds	
	D. F.	Mean square	D. F.	Mean square	D. F.	Mean square	D. F	Mean square
Total	546	182.17	686	66.15	109	86.37	127	43.97
Between litter sizes .	11	1764.05[1]	8	643.90[2]	—	—	6	103.43
Between months .	33	933.08	58	117.68	6	281.87	15	99.84
Between litters .	21	629.60[1]	94	186.39[2]	7	340.83[2]	13	64.37[1]
Within litters .	481	74.94	526	30.19	96	55.60	93	28.26

[1] $P < 0.05$; [2] $P < 0.001$.

VOL. 31 1950

within months. If the variance is divided into different parts according to the method developed by WINSOR and CLARKE (1940), the variance for "between months" amounts 13.5 % of the computed total variance for Large race and 13.7 % for LS-crossbreds. For the two other groups the variance is "negative".

c. AGE AND LITTER NUMBER.

A series of investigations into the birth weight in uniparous animals have shown that it rises with the increasing age of the mother. Essentially, this is the same as saying that the birth weight increases with the number of births. In particular, the weights of newborn calves have been examined, but as the size of the dam is to a certain extent dependent on her age, it has not in general been possible to distinguish between the influence of size and the influence of age. This applies for instance to investigations by MOSELEY *et al.* (1929) and HANSSON (1945). On the other hand, TYLER *et al.* (1947) found a partial correlation between calving number and birth weight of 0.26, and in other cattle breeds it has been found that the size of the calf is two and a half to five times more closely correlated with the physiological age of the mother than with her size within the calving number (VENGE 1949).

For such multiparous animals as pigs it was shown by OLOFSSON and LARSSON (1930) among others, that the birth weight is slightly less for pigs borne by young than by older sows. CARMICHAEL and RICE (1920) showed that the age of the sow has a perceptible effect on the weight of the newly born pigs. The difference between pigs borne by young and by older sows was 1/4 to 1/2 lb.

EATON (1932) could not show any correlation of importance between the birth and the age of the mother or the litter number in guinea pigs, whereas with rats, KING (1915) found a rather close relationship between these two variables. BLUHM (1929) was able to ascertain a fairly large age influence on the birth weight of mice in the first litter, but the age influence decreased gradually. For later litters the correlations between the birth weight and the age of the mother and between the birth weight and the litter number are of the same size, $r = 0.1$.

It was shown by KOPEĆ (1924 a) that in rabbits there is no difference between the birth weight of young by one or two year old does, whereas there is a tendency for young by mothers of three years or more to be heavier than the offspring of younger mothers. In the present investigations it has very seldom occurred that does more than two years old have been used. The relationship between the age of the doe and the average litter size and weight per young of does whose exact age has been known at the birth of the litter, are shown in Fig. 10.

On the basis of these data it is considered permissible to disregard the influence of the age and the litter number in ensuing calculations.

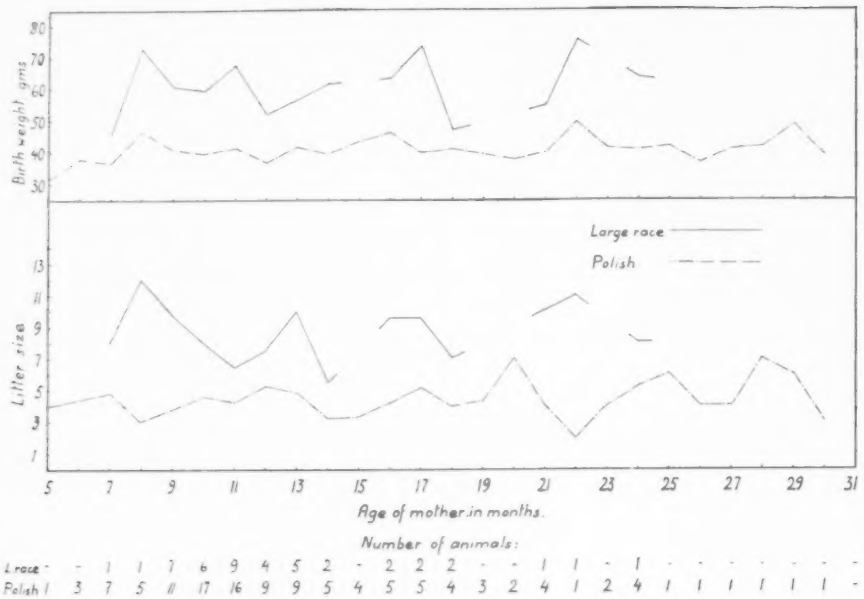

Fig. 10. Litter size and birth weight of the young in relation to age of doe. Large race and Polish.

VOL. 31 1950

d. SIZE OF THE MOTHER.

a. Effect on the total production of young.

In normal litters the size of the mother is positively correlated with litter size as well as with litter weight, whereas in some of the experimental groups a negative correlation has been found (Table 12). This may be due to the fact that quiet accidentally a smaller number of ova have been transplanted to the large does within an experimental group, or it may be a question of interaction between the fatness and the fertility of the mother animal. It is, however, only in the case of the Polish breed that there is a significant correlation ($P < 0.01$) and in the case of pure breeds borne by crossbreds a slight correlation ($P < 0.05$) between the weight of the mother and the size of the litter. With regard to the weight of the mother and the weight of the litter, the correlations are significant in both of the normal groups.

It has previously been mentioned (page 33) that there is a racial difference in the potential fertility, and the fact that there is also a real difference in the actual fertilitiy between races of different sizes has been stressed by several authors (KOPEĆ 1924 a, PICKARD 1930, HAMMOND 1934, GREGORY 1932, and others). Within races, KOPEĆ (1923, 1924 a) found a correlation between the physical condition of the mother and the weight of the newly born young, and

at the same time he showed that pregnancy itself had a "favourable" influence on the weight of the mother. It is thus a question of the interplay of influences. HAMMOND (1921) observed a marked positive correlation between the size of the mother and the size of the fetuses and concluded that the difference in the birth weight was partly due to hereditary differences, but that the nutrition of the mother also exerted a certain influence.

For the purpose of examining whether there has been a tendency to transplant fewer ova to larger does or *vice versa*, which might be the cause of the negative correlations between maternal weight and litter size, the percentage of young of transplanted ova within the transplantation litters has been calculated and correlated with the weight of the mother. The correlations are —0.0719 in the case of Polish and —0.2537 in Large race does. In neither case are the coefficients significant, but in spite of this it is not possible, so far as Large race is concerned, to disregard that an accidental selection may have taken place, with the result that the larger does have in fact received more ova than the somewhat lighter does. The correlation between weight of doe and number of ova transplanted is 0.2242 ($P < 0.2$) for Large race and 0.0578 for Polish ($P < 0.7$). At the same time the results indicate that the large does within a race-group, i.e. generally speaking the fatter animals, have not been such good receptors as the leaner animals.

β. Effect on the size of the individual young.

The factors discussed so far are such as may be supposed to act principally on the total production of young, i.e. the litter weight. However, for this in-

TABLE 12. Regression (b) of litter size and litter weight on weight of mother, and litter weight on litter size; and the corresponding coefficients of correlation (r).

Group	Number of animals	Litter size — weight of mother		Litter weight — weight of mother		Litter weight — litter size	
		r	b	r	b	r	b
AI. Normal Large race	56	0.2215	1.19	0.4039[2]	129.59	0.8465[3]	50.37
BI. Normal Polish . .	102	0.2672[2]	2.02	0.3474[3]	93.96	0.9209[3]	32.37
AII. Large race, transpl.	25	0.1087	0.73	0.2723	68.69	0.9317[3]	34.99
BII. Polish, transpl. . .	19	−0.0373	−0.76	−0.1715	−19.61	0.9602[3]	53.56
CII. SL-crossbreds, transpl.	27	−0.1599	−1.12	−0.0303	−8.61	0.8871[3]	35.98
DII. LS-crossbreds, transpl.	22	−0.3174	−1.15	−0.3421	−63.72	0.9294[3]	47.66
E. Pure breeds born by crossbreds . .	25	−0.4299[1]	−1.24	−0.3437	−56.54	0.9458[3]	53.80

[1] $P < 0.05$; [2] $P < 0.01$; [3] $P < 0.001$.

vestigations as a whole, the object of which is to determine the maternal influence on the size of the individual young, it is of greater interest to examine the extent to which the weight of the individual young is influenced by variations in the weights of the mothers within breeds. In the following discussion of the correlations between the size of the mother (kgms.) and the birth weight of the young (gms.), the individual groups are therefore treated separately. The calculations have been carried out in the form of analyses of co-variance.

*A*I. *Normal Large race.*

For normal young of Large race it has been possible to calculate the correlation between the weight of the young at birth and the weight of the mother from data for 273 young. The total correlation (Table 13) is 0.1566 ($P < 0.01$), in this case positive, just as the correlation between the weight of the mother and the weight of the litter, as has previously been mentioned. If calculated "within litter sizes", the two coefficients are of the same order, $r = 0.4868$ for maternal weight and weight of the young, and $r = 0.4341$ for maternal weight and weight of the litter. For this breed there is a real difference in the regression lines, both with regard to intercept and slope ($P < 0.001$), but if the material is grouped in such a way that litter sizes one and two are regarded as one group, litter sizes three, four, and five as another, and those above five as a third, the differences in slope are no longer significant. This indicates that within all litter sizes there is a similar relationship between the size of the mother and the size of the young.

*A*II. *Large race borne by Polish.*

Surprisingly, the total correlation between the size of the mother and the weight of the young is less pronounced in the case of Large race young borne by Polish does (Table 13), the total correlation being 0.1886 ($P < 0.1$). The most plausible explanation is probably to be found in the fact that, considering the weight differences which can be observed in Polish does, it is of minor importance whether the mother is a little larger or a little smaller than the average. Frequently differences in size will in reality be due rather to differences in the animals' state of health than to actual anatomical size differences. On the contrary it is probable that, just as in the case of Large race does, the fat does allow the fetuses a somewhat poorer possibility to develop than do the does whose fatness is in keeping with their size.

Within litter size there is a significant correlation between the weight of the mother and the weight of the young, with a coefficient of 0.4549 ($P < 0.001$). The size of this coefficient is quite in keeping with the correlation between the size of the mother and the weight of the litter, but the significant correlation

VOL. 31 1950

TABLE 13. Regression (b) of birth weight of the individual young on the weight of the mother; and the corresponding coefficients of correlation (r).

	Number of animals	Total		Within litter sizes	
		r	b	r	b
A_I. Normal Large race	273	0.1566[2]	3.64	0.4868[3]	12.81
B_I. Normal Polish	313	0.0247	1.00	0.1079	4.30
A_{II}. Large race, transpl.	78	0.1886	11.33	0.4549[3]	15.25
B_{II}. Polish, transpl.	64	−0.4115[3]	−5.12	−0.3865[2]	−6.26
C_I. Normal SL-crossbreds	83	0.1625	2.77	−0.0493	−0.94
D_I. Normal LS-crossbreds	67	0.2374	5 62	0.3246[1]	8.65
C_{II}. SL-crossbreds, transpl.	73	0.3303[2]	16.34	0.2382[1]	9.18
D_{II}. LS-crossbreds, transpl.	78	0.1146	2.54	−0.1604	−2.92

[1] $P < 0.05$; [2] $P < 0.01$; [3] $P < 0.001$.

between mother and young found in this case is in such contrast to the previous result that it might reasonably be presumed that other uncontrollable factors have tended to obscure the total correlation between the two characters. It seems as if the coefficients are influenced particularly by the litter size.

A comparison of the regression lines between the different litter sizes shows, as might have been expected, a highly significant difference ($P < 0.001$) between the intercepts of the lines. Between the individual litter sizes there is also a statistically significant difference between the slopes of the regression lines ($P < 0.01$), but this is attributable to the very steep regression line for litter size three and the equally steep but reversely directed line for litter size four, and it is doubtful if any real biological importance can be attached to the difference.

$A_I + A_{II}$. *Comparison of normal and transplanted Large race young.*

A study of the relationship between the weight of the young and the weight of the foster-mother, irrespective of whether the fetuses have developed in small or large mothers, shows that the variates do not deviate more from the parallel regression lines for normal and transplanted within litter size than might be expected by chance (Table 14). As a matter of fact only very limited data, in which both transplanted and normally born litters are represented within the same litter size, is available for this analysis, and in consequence the computed regression lines are rather unreliable. The fact that there is no difference in the regression lines does not by any means exclude the possibility of significant differences in the size of the young within the same litter size. This will be dealt with subsequently.

TABLE 14. Analysis of regression of individual birth weight on weight of doe. Comparison of normal and transplanted.

Source of variation	$A_I + A_{II}$. Large race			$B_I + B_{II}$. Polish			$C_I + C_{II}$. SL-crossbreds			$D_I + D_{II}$. LS-crossbreds		
	D.F.	Remainder mean square	Variance ratio	D.F.	Remainder mean square	Variance ratio	D.F.	Remainder mean square	Variance ratio	D.F.	Remainder mean square	Variance ratio
Total	349	186.49		375	92.32		154	110.54		143	80.67	
Differences in intercepts of regressions for litter sizes	11	2861.41	28.78[3]	7	731.04	9.12[3]	11	721.21	11.35[3]	6	656.27	12.58[3]
Remainder	338	99.43		368	80.18		143	63.56		137	52.39	
Differences in slopes of regressions for litter sizes	11	771.33	10.04[3]	7	270.86	3.54[2]	11	152.08	2.71[2]	6	68.43	1.32
Remainder	327	76.83		361	76.48		132	56.19		131	51.65	
Differences in intercepts of regressions for normal and transplanted	4	174.76	2.31	7	533.65	7.91[3]	2	—	—	5	153.51	3.22[2]
Remainder	323	75.62		354	67.44		130	—		126	47.61	
Differences in slopes of regressions for normal and transplanted	4	18.35	—	7	155.33	2.37[1]	2	—	—	5	81.15	1.76
Error	319	76.34		347	65.66		128	—		121	46.23	

[1] $P < 0.05$; [2] $P < 0.01$; [3] $P < 0.001$.

VOL. 31 1950

B_I. *Normal Polish.*

Data for 313 young is available for the computation of the correlation between the maternal weight and the birth weight of the individual young. The results are compiled in Table 13. The total correlation is 0.0247 ($P < 0.7$), but within litter sizes there seems to be a closer correlation between the two variables, viz. 0.1079 ($P < 0.06$). There is a highly significant difference between the regression lines for each litter size, and likewise the slopes of the regression lines for the individual litter sizes differ significantly ($P < 0.001$). A more detailed study of the individual regression coefficients does not, however, disclose any definite trend, and it would be hazardous on the basis of these figures to draw the conclusion that the size of the young is materially influenced by the size of the mother within the range studied here. The mean regression coefficient within litter size is computed at 4.30 gms.

B_{II}. *Polish borne by Large race.*

The relationship between the maternal weight and the birth weight of Polish young developed in Large race does differs entirely from the foregoing. For this group a negative total correlation for 64 variates of -0.4115 ($P < 0.001$) is found, and the mean correlation within litter size is -0.3865 ($P < 0.01$) (Table 13). This is partially explained by the fact that a couple of purebred Flemish Giant does bore small litters of very low weights. It is, however, of far greater interest that it is not possible to show any difference either in the intercepts or the slopes of the regression lines between the individual litter sizes, and this can only be accounted for if it is presumed that the intra-uterine environment provides sufficient space to ensure the development of the Polish fetuses to the genetically determined maximum size, irrespective of whether there are few or many individuals in the litter. Within the individual litter sizes the regression coefficients are negative, as is the case of the mean coefficient. It is difficult to explain these negative regressions unless they are associated with the obviously poor constitution of the very large Flemish Giant does; but it is impossible on the basis of this material to reach a definite conclusion.

$B_I + B_{II}$. *Comparison of normal and transplanted Polish young.*

A statistical treatment of the data for the two preceding groups when pooled merely confirms the obvious difference which exists between Polish young developed in Polish or Large race does. The analysis (Table 14) has been carried out in such a way as to permit a comparison of the differences between the various regression lines within litter sizes, and it will be seen that the difference between the intercepts of the lines is highly significant ($P < 0.001$),

whereas the difference between the slopes of these lines is scarcely so pronounced, although it is still statistically significant ($P < 0.05$). The joint regression lines within experimental groups, i.e. transplanted—normal, have a very slightly negative trend ($b = -2.26$), but no real importance can be attached to this. The mean correlation coefficient within the same groups is 0.0818 ($P < 0.15$) and is thus not indicative of any close connection between the weight of the mother and the size of the young, as has already been pointed out in the discussion of the normal material.

C_I. *Normal SL-crossbreds.*

Unfortunately the data for crosses between Polish buck and Large race doe, from which details about the maternal weight can be drawn, does not comprise more than 83 young distributed between eight different litter sizes. This results in low precision, and the correlation coefficients which have been found are not significant (Table 13). The total correlation is slightly positive, and the correlation within litter sizes between maternal weight and weight of the young is so insignificantly negative that it is justifiable to suppose that it does not differ from zero. In two cases in which are several litters within the same litter size, there is a difference between the intercepts of the regression lines which is significant ($P < 0.01$).

VOL.
31
1950

C_{II}. *SL-crossbreds borne by Polish does.*

The data for the SL-crossbreds borne by Polish does is of the same order of size as for the normal material. It comprises 73 young in 27 litters, distributed between six different litter sizes. The total correlation (Table 13) between maternal weight and weight of the young is again positive, $r = 0.3303$ ($P < 0.01$), and likewise there is a significant positive correlation within litter sizes of 0.2382 ($P < 0.05$). As was found in the case of Large race young borne by Polish does, there is in this experimental group also a significant difference between the regression lines, both with regard to the intercepts and slopes. The regression coefficients for litter sizes one and two are negative, but the other litter sizes show a positive regression, so that in this case there appears to be a difference between pure breeds and crossbreds, all borne by Polish. It is difficult to find a biological explanation of this discrepancy, and it would therefore appear most probable that it is merely to be attributed to chance, unless here again there is the influence of fatness.

$C_I + C_{II}$. *Comparison of normal and transplanted SL-crossbreds.*

Because of the uneven distribution of the litters within litter sizes, a treatment of the pooled data for both groups gives the anomalous result that it is

impossible to test the difference between the regression lines for normal and transplanted young within litter size (Table 14). The difference between the intercepts and slopes of the lines for the different litter sizes is of little or no interest in this connection.

D_I. *Normal LS-crossbreds.*

The normal material for which details are available consists of 67 young, borne in five different litter sizes. The total correlation, $r = 0.2374$, is not significant ($P < 0.1$) (Table 13), whereas the correlation between maternal weight and weight of the young within litter sizes, $r = 0.3246$, is significant ($P < 0.05$). There is a highly significant difference between the intercepts of the lines ($P < 0.001$), but the difference between the slopes of the lines is very slight, although significant ($P < 0.05$).

D_{II}. *LS-crossbreds borne by Large race does.*

Data comprising 78 young borne in 22 litters of seven sizes is available for transplanted fetuses. There is no apparent relationship between the weight of the mother and the weight of the young, the total correlation being small and positive, but not significant, $r = 0.1146$ ($P < 0.4$) (Table 13). The correlation within litter size on the other hand is slightly negative, $r = -0.1604$ ($P < 0.2$), and has the same slope as the regression found in Polish young borne by Large race. The intercepts of the regression lines within litter size are significantly different ($P < 0.001$), but there is nothing to indicate that the variates for the individual litter sizes deviate more from the parallel regression lines for each individual litter size than can be attributed to chance.

$D_I + D_{II}$. *Comparison of normal and transplanted LS-crossbreds.*

Pooling the normal and transplanted groups (Table 14) shows a significant difference between the intercepts of the regression lines, calculated within litter sizes, for these two groups ($P < 0.01$). There is no significant difference between the coefficients of regression. It will be noted that, as for the other groups, there is a highly significant difference between the intercepts of the regression lines for the different litter sizes ($P < 0.001$).

e. SEX.

A question that is of great interest in connection with these investigations is whether there is a difference between the two sexes with regard to the birth weight. KOPEĆ (1925) observed a slightly higher birth weight for males than

TABLE 15. Mean birth weight of males and females for the normal groups.

Litter size	Large race				Polish				SL-crossbreds				LS-crossbreds			
	♂♂		♀♀		♂♂		♀♀		♂♂		♀♀		♂♂		♀♀	
	n	Weight, gms.	n	Weight, gms.	n	Weight, gms.	n	Weight, gms.	n	Weight, gms.	n	Weight, gms.	n	Weight, gms.	n	Weight, gms.
1			2	109.0	1	64.0	3	47.7					1	32.0		
2			—	—	11	44.3	11	45.2			2	76.5	1	52.0	1	58.0
3			—	—	22	45.3	26	45.6	1	62.0	2	60.5	14	47.1	13	49.5
4			—	—	32	42.4	32	41.3	—	—	—	—	20	46.8	8	49.3
5	6	64.0	9	77.1	43	42.1	37	40.5	3	56.3	2	58.0	8	50.0	7	50.0
6	13	59.5	11	54.9	39	41.2	39	39.6	4	60.3	2	57.5	—	—	—	—
7	8	55.0	6	57.2	19	39.1	16	36.7	4	50.5	3	54.7	4	47.5	3	41.3
8	19	54.5	21	54.3					—	—	—	—				
9	14	53.6	13	51.2					6	46.2	3	41.3				
10	20	56.8	10	53.8					—	—	—	—				
11	12	51.9	10	47.9					—	—	—	—				
12	13	45.4	11	47.4					—	—	—	—				
13									7	46.3	6	41.3				

n = number of animals.

VOL. 31 1950

for females in large litters and the reverse in small litters, but the difference does not appear to be significant. The birth weights for males and females of normal breeds are shown in Table 15. From this it is seen that in the case of Large race there does not appear to be any definite trend, for in four cases the mean is higher for females, while in other four cases the males have the higher birth weight. An analysis of variance (Table 16) shows a significant difference between the sexes in Large race ($P < 0.001$). The computed variance amounts to 12.9 per cent of the total, but as is shown in Fig. 11, there is very great variation between the individual litters and litter sizes. The figures for Polish show a more consistent behaviour, although the differences within litter sizes are but small and insignificant. Here the computed variance amounts to 5.8 per cent of the total variance. For the normal crossbred groups no variation between the sexes has been found in any case which indicates a difference between them with regard to the birth weight. In both cases the calculated variance is "negative".

On the basis of these results it is considered valid to leave differences between the sexes out of consideration in the ensuing calculations. The legitimacy of this procedure will become more apparent from results to be presented later, when it will be shown that the whole of the variation between the sexes is within the individual litters. Moreover, as no statistically significant differences between the frequency of males and females within the various groups have been ascertained, it is improbable that any differences in the weight of the sexes at birth will be able to affect the final results.

f. LITTER SIZE.

A very important factor in the determination of the birth weight in multiparous animals is the litter size. For mice, BLUHM (1929) found a correlation between litter size and birth weight of —0.37, but stated that the reason

TABLE 16. Difference in birth weight between males and females for the normal groups.

Source of variation	Large race		Polish		SL-cross-breds		LS-cross-breds	
	D. F.	Mean square	D. F.	Mean square	D. F.	Mean square	D. F.	Mean square
Total	197	151.45	330	57.36	44	110.10	79	40.05
Between litter sizes .	8	1615.85[1]	6	317.09[1]	6	487.23[3]	5	93.36
Between litters . .	17	532.16[3]	74	145.53[3]	—	—	16	76.79
Between sexes .	24	115.46[3]	62	30.89	6	17.06	16	23.61
Within sexes . .	148	34.40	188	23.10	32	56.84	42	25.97

[1] $P < 0.05$; [2] $P < 0.01$; [3] $P < 0.001$.

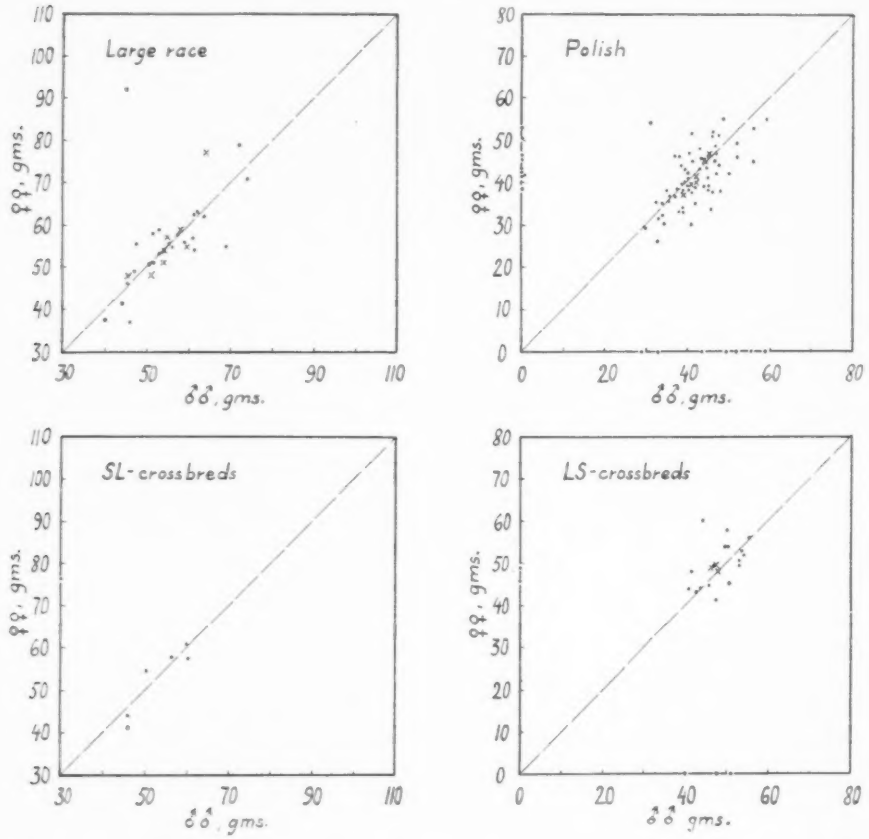

Fig. 11. Difference in birth weight between males and females. A dot represents the mean weight of males and females respectively within a litter, a cross designates the mean weight of all males and females in litters of a certain size.

for this low value was that, on the average, singly born young had a weight that was below the weight of both twins and triplets. Another reason for the comparatively low correlation is probably to be found in the fact that it has been calculated on the assumption of a linear relationship whereas the relationship between the two factors seems in fact to be curvilinear. This has been emphasized by ENZMANN and CROZIER (1935), who came to the conclusion that the increase in the litter weight due to a larger number of young in the litter is directly proportional to the total litter weight, but inversely proportional to the number. This is expressed by the formula: $\frac{\triangle W}{W} = k \cdot \frac{\triangle N}{N}$, which can be rewritten $W = N^k + C$, where W is the litter weight, N is the number and k and C are constants. WRIGHT (1922) showed that the correlation between

VOL. 31 1950

litter size and birth weight was —0.66 for guinea pigs, and EATON (1932), who likewise worked with these animals, found a correlation of a similar order for the total material, but by keeping the maternal weight constant the coefficient became —0.79. In rabbits, HAMMOND (1921), WISHART and HAMMOND (1933), and KOPEĆ (1923) found a decrease in the birth weight of the individual young with an increase of the number of young in the litter, but in no case did they examine whether the regression is linear or not.

In the case of pigs it is known that the birth weight is strongly influenced by the litter size (CARMICHAEL and RICE 1920, MCKENZIE 1928, OLOFSSON and LARSSON 1930, LUSH *et al.* 1934, OLSEN 1939) in a similar way to that which has been ascertained for mice, guinea pigs, and rabbits, and to some degree for rats (KING 1915). The conclusions reached by HALL and PIERCE (1934) from experiments with cats are in conflict with these, for they found a pronounced maximum value for litters of five. SIMPSON (1913) working with dogs, although only with limited material, arrived at the opposite results, for the minimum weight was found in the average litter size. KING's results of experiments with rats (1915) are of interest, because she found a smaller average birth weight in litter sizes below five than in those above, but by dividing the mothers of the small litters into two groups according to their condition, the results are found to be in agreement with those of the investigations already discussed. Further, it can be mentioned that GATES (1925) found very high "birth weights" in twenty-four hour old mice from small litter sizes and decreasing weights in larger litters, a result in keeping with what was later shown by BLUHM (1929). IBSEN (1928), working with guinea pigs, found, in accordance with WRIGHT (1922), that a perceptible decrease in the birth weights accompanied an increase of the number of young in the litter, and at the same time he ascertained that the correlation between the weight of the young and the weight of the mother increases as the weight of the young decreases. Several of these investigations have revealed that the birth weights of singly born or twin-born young are on the average lower than the weights in litters of three or more. This applies, for example, to BLUHM's experiments with mice, HAMMOND's (1921) with rabbits, and to most of the above mentioned investigations into the birth weights of pigs, (e.g. LUSH *et al.* 1934). These observations will not be discussed further in this connection, although it is considered that these small litter sizes are due to abnormal, perhaps even to pathological conditions in the uteri of the mothers, for it is not difficult experimentally to obtain higher birth weights for singly born or twin-born young developed in sound mothers.

From the above review it would appear that in multiparous animals the birth weight of the individual young is generally greatly dependent on the number of young in the litter, and that the correlation between the two factors is distinctly negative. In agreement with these views, the correlations between

the birth weight of the individual young and the litter size found in the present investigations are significantly negative as is shown in Table 17.

In the calculation of these coefficients, the weight of each individual young is correlated with the size of the litter to which it belongs. By this method the influence of the large litters will be given undue weight at the cost of the small litters, as will also be the case when the birth weight is correlated with the maternal weight. As has previously been mentioned, it must be presumed that it is the total litter weight which influences the gestation period, since it is most improbable that the weight of the individual young should be able to affect this except in connection with the litter size. It would therefore appear preferable to base the calculations on the total production of young by combination of these correlation coefficients.

In contrast to what is found for the birth weight of the individual young, there is, as would be expected, a positive correlation between the litter weight and the litter size of all the groups as a whole (Table 12). In all cases, very large and statistically significant correlations have been calculated which indicate an almost linear relation between the two characters within the intervals covered by the calculations. In spite of this it would be completely unreasonable to suppose that this really should be the case, for if conversely the mean birth weight is made the basis of the calculations a negative correlation or regression is found. Within the range of observed litter sizes, this is in full accordance with the observations. However, by extrapolation, negative weights of the young should occur in litters of ten to fourteen (depending on the breed of the mother), a result which is absurd. For Large race borne by Polish the regression equation is $y = 85.03 - 7.24\,x$. The mean birth weights observed and the calculated weights per young are shown below.

Birth weight for Large race borne by Polish.

Litter size	Observed	Computed
1	77.8	77.8
2	70.4	70.5
3	65.3	63.3
4	55.5	56.1
5	47.9	48.8
6	42.1	41.6

It is seen that there is very good agreement for litter sizes from one to six. In this case the correlation is -0.8355 ($P < 0.001$). However, if the number of young in the litter were increased to twelve, which is by no means unreasonable even though the largest number of young found in a litter in the normal material is nine, the birth weight should, by extrapolation, be -1.91 gms.

It now becomes necessary to fit regressions to the data for birth weight

VOL
31
195

TABLE 17. Correlation between litter size and birth weight of the individual young.

Group	Number of animals	r
A_I. Normal Large race	547	-0.3313^3
B_I. Normal Polish	687	-0.2968^3
A_{II}. Large race, transpl.	78	-0.8355^3
B_{II}. Polish, transpl.	64	-0.2120
C_I. Normal SL-crossbreds	110	-0.5066^3
D_I. Normal LS-crossbreds	133	-0.2591^2
C_{II}. SL-crossbreds, transpl.	73	-0.7420^3
D_{II}. LS-crossbreds, transpl.	78	-0.6199^3
E_1. Large race born by crossbreds	40	-0.5868^3
E_2. Polish born by crossbreds	35	-0.3884^1

^{1} $P < 0.05$; ^{2} $P < 0.01$; ^{3} $P < 0.001$.

VOL. 31 1950

(or conversely, litter weight) and litter size, which functions should also comply with certain biological requirements. The first requirement is that the function should approach zero with increasing litter size without, however, becoming zero. Further, it is required that the function does not show any fetus weight in the case of no young in the litter, or expressed in another way: If the number of young approach infinity, the fetus weight ought to approach zero (and if the number of young approach zero the fetus weight ought to approach infinity). However, in addition to compliance with these two purely biological requirements, there is a third and very important restriction, if such a function is to have any value, it must be easy to handle. Following ENZMANN and CROZIER (1935) the view is here adopted that the increase in the litter weight accompanying an increase in the number of young in the litter is proportional to the weight and inversely proportional to the number. Thus: $\frac{dy}{dx} = \frac{y}{x} \cdot k$ or $y = C \cdot x^k$, which formula (known as HUXLEY's relative growth function, 1932), satisfies the requirement of simplicity. Then reversing the function so that the birth weight decreases proportionately to the increase in the litter size it becomes $y = C \cdot x^{-k}$, which satisfies the three requirements. Another simple function, which does not, however, quite comply with the requirements, is the ordinary growth function $y = ae^{-kx}$, where a is a constant ($= y_0$) and k is the proportionality factor. The function implies that the decrease in the birth weight (—dy) is proportional to the increase of the number of young in the litter (dx). Both of these functions have been examined for the various normal and transplanted groups and will be discussed in detail under the appropriate headings.

A question of very great interest in connection with the litter size is the extent to which the variation within the litter in the case of each individual

litter size increases on an increase of the number of young or whether the variation within the litter remains constant irrespective of the presence of many or few young in the litter. Various theories have been advanced as to the influence which the position of the fetus in the uterus has on the size. KREIDL and NEUMANN (1911) examined the size of the embryos at different stages of the pregnancy in dogs, cats, guinea pigs, mice, rats, and rabbits and asserted that the heaviest fetus was most frequently to be found in the distal (tubal) part of uterus, and that the other fetuses in the same uterus "ranged themselves" according to weight, the lightest fetus generally being found proximally (vaginally). Unfortunately the data was not subjected to statistical analysis, and as only about one half of the material relating to rabbits consists of fetuses sufficiently advanced in development that the weight differences found can be supposed to have existed until after the birth, it is difficult to draw any sound conclusions regarding the variation within individual litters. However, ROSAHN and GREENE (1936) came to the result, likewise for the rabbit, that the fetus developed in the upper part of the uterus had a higher weight than the other fetuses in the corresponding horn, and that the weight decreased with the order of implantation. An exception was found in the cases where the uterine horn contained only two fetuses. In this instance no significant influence of the position on the fetal weight could be shown. A progressive decrease in weight due to position in uterus was also found for the placentae and there was a highly significant correlation between fetal weight and placental weight. HAMMOND (1921) showed that the uterine horn which held the largest average number of fetuses had the higher weight of fetuses (not significant), but at the same time it was ascertained that the heaviest fetus appeared rather more frequently in the uterine horn that had received the smallest number of implanted fetuses. The conclusion is that the weight or size which the fetus reaches is independent of the number to be found in the same uterine horn, just as the size of the fetal membranes is to a far greater extent dependent on the viability of the individual fetus than on the uterine environment. This is a most interesting observation which will be discussed later in connection with the results of the individual transplantations, but if the hypothesis is true, it means that the size differences which are found within litters of the same size must for the greater part be regarded as being of a genetic nature, whereas differences between the individual litter sizes (in so far as the litter size is not genetically conditioned) would seem to be rather more dependent on the quantity of nutrient available to the individual fetus.

Of course it has not been possible to control these factors in the present investigations and it is only by means of statistical methods that it has been possible to determine whether the variation between litter sizes is constant. The data for the different breeds may then be compared. The results are shown in Table 18, from which it appears that there is no difference whatever in the

VOL
31
195

TABLE 18. Difference in coefficients of variation for birth weights of individual litter.

Source of variation	Normal groups		Transplanted groups	
	D. F.	Mean square	D. F.	Mean square
Total	262	48.33	75	30.94
Between breeds	3	33.35	3	92.86
Between litter sizes	31	44.43	18	29.63
Within litter sizes	228	49.05	54	27.93

coefficients of variation for the individual litters, either between breeds or between litter sizes. The absolute standard deviations of course differ from breed to breed, as for example between Polish (S. D. = 4.68) and Large race (S. D. = 7.80) where the absolute variation is 1 2/3 as great for the latter as for the former, but the relative variation, as expressed in the coefficients of variation, is of the same order if the litter sizes which are represented by only one or a few litters are left out of consideration. The mean coefficients of variation is as follows:

	Normal		Transplanted	
	Number of litters	C. V., %	Number of litters	C. V., %
Large race	64	12.53	19	11.01
Polish	152	11.95	18	7.88
SL-crossbreds	14	12.97	20	5.70
LS-crossbreds	33	11.56	19	7.85

Consequently the whole of the existing variation is due to a difference between the individual litters, and within the scope of this analysis there is therefore nothing which is in direct disagreement with HAMMOND's observations. It seems then justifiable to draw the conclusion that by far the most important cause of size differences between litter mates must be found in genetic variations. However, it would be hazardous to ascribe the whole of the variation to genetic differences between individuals of the same litter, for part of it is presumably attributable to anatomically conditioned differences in the supply of blood to the uterine organs of the mother individual, which in turn may cause an uneven distribution of the nutrients available for the embryos. Further, part of the variation is perhaps due to an age difference between the fetuses (WATERMAN 1943). In general, however, the time that passes between the births of the first and the last fetus is very short, and experience has shown—in agreement with KOPEĆ's observations (1924)—that it would be wrong to suppose that the young born first would have time to take nourishment before the last fetuses were born. If such a factor were operative, it would tend to increase the variation coefficients between litter sizes, but, as is shown in Table 18, no variation has been found "between litter sizes".

g. LIVEBORN AND STILLBORN YOUNG.

The causes of stillbirths have been the subject of a good deal of speculation, although it cannot be said that the phenomenon is well understood. Some of the causes are known, however, e.g. lethal factors, pronounced dystocia, and purely pathological disturbancies. On the other hand, there are numerous cases in which it is very difficult indeed to find any plausible explanation of the appearance of dead individuals among otherwise healthy and viable litter mates. In such cases the explanation is frequently desorted to that death is due to suffocation during the parturition. GRÜNEBERG (1943) asserted that the majority of stillbirths in mice are caused by the physiological condition of the mother individual, and this is quite probably the correct explanation in a number of cases in these present investigations. It was shown by MURRAY (1934) that the percentage of stillborn fetuses rises as the age of the mother increases, but at the same time it must be emphasized that a different genotype gives a different percentage of stillbirths (GREEN 1930). Thus the more viable cross-breds have a lower percentage of stillborn fetuses than the pure breeds. This indicates that the genetic constitution of the fetus itself is of equally great importance for the viability. As the physiological condition of the mother must be supposed to have approximately the same influence on each individual in the litter, it is reasonable to suppose that this characteristic of the fetus is most important. If the physiological conditions which are offered to each individual fetus should be of decisive importance, it would mean that the mortality rate of the fetuses would be higher in the large litters than in the small, but, as stated by HAMMOND (1921), this does not seem to be the case for rabbits. HAINES (1931) showed for guinea pigs that the size of litter bore an important relation to mortality of the young at birth. Another possibility, which must also be taken into consideration, is that there are in fact two or more interacting causes of mortality. This is borne out by the results of the transplantation experiments on Large race fetuses borne by Polish does. In five out of six litters of only one young the fetus has been too big to be born alive. Obvious physical injuries have been observed, a heavy constriction and a partial distention of the bones of the cranium having taken place which may have caused suffocation and in some cases are observed to have caused internal hemorrhage. A constriction not accompanied by a hemorrhage would in itself not be able to cause the death of the fetus. Further, a stretching of the whole fetus has taken place, accompanied by an abnormally heavy dislocation of the viscera right down to pelvis. An autopsy showed a cavity between the liver and the stomach, immediately below the diaphragm, with the intestines pressed towards the rear. The same conditions, though less pronounced, have been observed for litter size two within this experimental group, in which eight out of fourteen fetuses were stillborn. In two litters both the young were born

VOL
31
195

alive, whereas in three litters both of them were stillborn. In two litters there have been one liveborn and one stillborn young. Therefore, in small litter sizes, where as a rule the young are larger than the average, the purely mechanical cause seems to be the predominant factor, whereas in large litters the physiological cause is probably predominant.

On the basis of the comparatively limited material it is of course impossible to draw any real conclusions concerning the cause of mortality. However, no injuries of the fetuses have been observed in the larger litters. It is therefore most probable that in these cases the mortality is caused by the same, unknown, factors as in offspring by normally mated does. On the other hand it is not entirely impossible that the somewhat larger fetuses severely tax the constitution of the mother and their rapid growth during the last part of the gestation period (HAMMOND 1935, WATERMAN 1943) is incompatible with the size of the mother. As a result the rate of mortality is high for fetuses of "alien race" borne by Polish. Of Large race fetuses 38.5 per cent have been stillborn, and of the SL-crossbreds 23.6 per cent.

OL.
31
950

In some cases it has been observed that the weight of the stillborn young was lower than that of the others. This may be due to several causes, one possibility being that the genetically weakest fetuses, which are also the smallest, have a greater mortality during the parturition itself than the large and vigorous young. Further, the possibility of fetus mortality some days before the parturition must also be taken into consideration, and if such is the case, the reason for the smaller weight must be attributed to this. If these fetuses are placed at the upper end of uterus they cannot be born until the living fetuses have completed their growth period and the parturition has started, as mentioned by WILLIAMS (1943). The death of a fetus at an earlier stage will probably result in resorption.

The analysis in Table 19 shows the difference between the regressions of the birth weights of liveborn and stillborn transplanted Large race and SL-crossbred borne by Polish does, on the litter size. It is seen that there is a significative difference between the intercepts and slopes of the regression lines for the two groups ($P < 0.01$). Further, there is a highly significant difference between the intercepts of the regression lines for liveborn and stillborn young ($P < 0.001$), but there is no difference in slopes. (The regression lines for liveborn are $y = 83.75 - 6.79\ x$ for Large race, and $y = 75.49 - 5.41\ x$ for SL-crossbreds, and for stillborn are the regression lines, $y = 86.94 - 8.04\ x$ for Large race and $83.10 - 10.43\ x$ for SL-crossbreds.) In other words, the variates, i.e. the individual young, do not deviate more from their own regression lines than can be attributed to chance. However, even if the lines for liveborn and stillborn young are parallel to the joint line for either of the two breeds, there is a significant difference between the intercepts of these two regression lines within either of the two breeds. This analysis therefore shows

TABLE 19. Analysis of regressions of birth weight of liveborn and stillborn on litter size.

Source of variation	D. F.	Remainder mean square	Variance ratio
Total	149	71.55	
Differences between the intercepts of the regressions for the two groups	1	562.44	8.24[2]
Remainder	148	68.29	
Differences between the slopes of the regressions for the two groups	1	430.88	6.86[2]
Remainder	147	62.83	
Differences between the intercepts of regressions for liveborn-stillborn within groups	2	597.40	10.77[3]
Remainder	145	55.46	
Differences between the slopes of the regressions for liveborn-stillborn within groups	2	151.67	2.80
Error	143	54.11	

[2] $P < 0.01$; [3] $P < 0.001$.

a statistically significant difference between the birth weights of the two categories of fetuses.

It would be interesting to compare the corresponding data for normal young of the pure breeds, but because of the nature of the material, a similar statistical analysis has not been carried out. In the years prior to 1946 no general weighing and sex determination of the stillborn fetuses were made, and it is possible to form an estimate of the weights only for limited material. These have been entered in Table 20. The number of young for which information is available is very small, and the figures must therefore be interpreted with caution. On the whole the weight of the stillborn young amounts to only about 80 to 85 per cent of the weight of the liveborn young. It does not seem that Polish fetuses differ from Large race fetuses with regard to viability, although there is a small discrepancy. In 169 litters of the former race a mortality of 8.60 per cent was found and in 70 litters of Large race the figure was 9.60 per cent.

The fact that the mortality among alien young borne by Large race and crossbred does is almost negligible, is very strong evidence in support of the assertion that the size of the fetus itself is of decisive importance for its viability. Within these two groups deaths have occurred in very few instances. In the case of Polish developed in Large race one stillborn litter of four, or 6.25 per cent, has been recorded. LS-crossbreds, likewise borne by Large race does, show two cases of stillborn young out of a total of 78, or 2.56 per

VOL.
31
195

cent. The figure for Pure breeds borne by crossbred does is 8.00 per cent of 75 young, for crossbred borne by crossbred does are four young stillborn of a total of twenty-one. These figures are probably not significantly different from the figures for normal Large race and Polish. For the normal crossbred groups the percentage of stillborn fetuses is 8.76 for 137 LS-crossbreds within 36 litters, and 6.09 per cent for 115 SL-crossbreds within 14 litters. It is not possible here to form an estimate of the distribution of the weights of the stillborn in relation to the weights of the liveborn young, but there seems to be a tendency to a slightly lower weight in the case of the former, just as there is a tendency to higher mortality among crossbred young by Large race buck and Polish doe. Once again this is evidently attributable to the fact that the fetuses are inclined to grow too large in proportion to the mother.

As it has not been possible in these preliminary investigations to show any significant difference between the normal breeds with regard to the fetus mortality at birth, and as further it seems most likely that it is the size of the fetus itself which is the decisive factor, provided no pathological conditions exist in uterus, then there is no reason to exclude the data for stillborn young from the computations. Therefore the results given are based on all individuals born, for which birth weights are available.

OL.
31
950

h. DISCUSSION OF THE FACTORS INFLUENCING BIRTH WEIGHT.

It has been shown in the preceding sections that a series of factors influence the birth weight, and that these factors vary in their importance. Further, it

TABLE 20. Weight of stillborn.

Born in litter size	Normal groups				Transplanted groups			
	Large race		Polish		Large race		SL-crossbreds	
	n	weight	n	weight	n	weight	n	weight
1	1	116.0	1	50.0	5	75.4	4	68.5
2	—	—	2	38.5	8	73.3	6	58.5
3	—	—	4	31.0	—	—	1	44.0
4	—	—	7	32.4	—	—	6	41.3
5	6	62.0	3	38.7	11	45.8		
6	—	—	6	32.2	6	39.3		
7	5	56.0						
8	8	51.0						
9	8	52.6						
10	4	50.3						
11	11	44.6						
12	3	57.0						

n = number of young.

appears that several of these "intrinsic" factors are to a limited extent and some "extrinsic" factors are to a very large extent, interdependent, for which reason the total correlation does not give a clear picture of the extent of the influence of each individual factor. The calculation of the partial correlations offers a means of separating these influences. A survey of the results thus obtained follows.

This discussion is limited to the material for which complete information is available, as it has been considered most expedient to use the same number of variates for the various correlation calculations. A question which is very difficult to decide in this connection, is whether it is justifiable to use the individual fetus weights, or whether it would be more correct to work with litter weights. Generally, the use of either would lead to the same result, except that in the case of the relationship between litter size and fetus weight the sign of the coefficient is reversed if the litter weight is used as the basis of the calculations. Further, the effect of variation within the litter could not be separated. Nevertheless, the best criterion seems to be litter weight. This procedure ensures that the gestation period and the maternal weight are given their proper values in the analysis, for otherwise they would be multiplied by the number of young in the litter.

VOL. 31 1950

1. General survey.

A high positive correlation between the litter weight and the litter size has been found in all groups, and it is probable that the litter size is the most important of all non-genetic factors which influence the weight of the litter at birth. This has already been emphasized in previous investigations, e.g. by EATON (1932), ENZMANN and CROZIER (1935) and others. This is obviously a one way correlation (path coefficient) as the litter weight at birth is dependent on the number of blastocysts implanted and not *vice versa*. It is therefore correct to speak of the litter weight (or the birth weight of the young) as a function of the litter size. The litter size in its turn is correlated with the weight of the mother, and as in these investigations it is the weight of the mother at the time of mating which is used in the calculations this correlation too is directionally determined. The weight of the mother is also correlated with the length of the gestation period, and this latter with the litter weight, but in this case it is to be presumed that the correlation found is the resultant of at least two components. It is quite conceivable that a high litter weight causes such an acute tension in the uterus that a shortening of the gestation period results, while at the same time it can be assumed that larger fetuses and consequently larger litter weight are themselves to some extent due to a longer gestation period. The correlation between the size of the litter and the length of the gestation period is strongly negative, and this too is in all

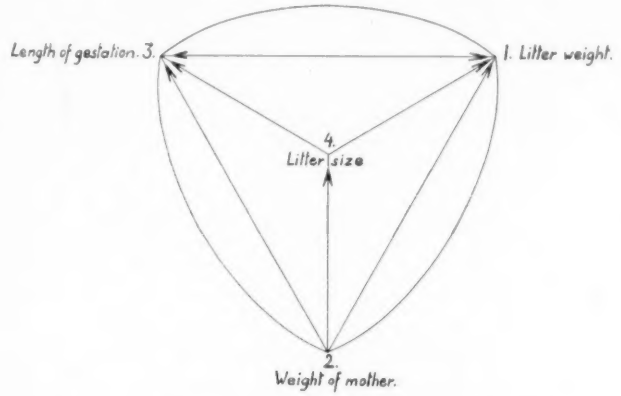

Fig. 12. Diagram showing the relationship between litter weight, weight of mother, length of gestation and litter size.

probability a one way correlation. Starting from the considerations just discussed the following scheme (Fig. 12) is drawn up to show the way in which the most important factors influence the birth weight.

The straight lines show the total correlation between two quantities, while the curved lines show the partial correlations which have been found by the analysis of covariance, i.e. the correlation within litter sizes.

2. Special survey.

It is not possible to give a detailed description of each of the individual groups which have been examined. The coefficients for the qualities examined have been entered for both normal and transplanted litters in Table 21. Designations such as ${}_{w}r_{12 \cdot 3}$ refer to the method used for calculating the partial correlation within litter sizes. It will be seen from the synopsis that the litter size is kept constant in all cases, whereupon the partial correlation has been calculated for, respectively, litter weight (1) — maternal weight (2) with the period of gestation (3) constant, litter weight (1) — period of gestation (3) with the maternal weight (2) constant and finally, maternal weight (2) — period of gestation (3) with the litter weight (1) as the constant factor (the last-mentioned calculation for the sake of completeness). It appears from the synopsis that, next to the litter size, the maternal weight is the factor which in Large race exerts the greatest modifying influence on the birth weight. The coefficient of 0.44 is significant ($P < 0.01$). In Polish there is no significant influence of the maternal weight on the litter weight if the influence of the litter size is eliminated and the length of the gestation period kept constant.

The length of the gestation period is but slightly related to the litter weight,

TABLE 21. Partial coefficients of correlation, within litter sizes.

Group	Number of animals	Litter weight -weight of mother	Litter weight -length of gestation	Weight of mother-length of gestation
		$w^r_{12 \cdot 3}$	$w^r_{13 \cdot 2}$	$w^r_{23 \cdot 1}$
AI. Normal large race	56	0.4448[2]	0.1089	−0.2715
BI. Normal Polish	102	0.0156	0.1925	0.1248
AII. Large race, transpl.	25	0.5338[1]	0.1510	0.0274
BII. Polish, transpl.	19	−0.3665	0.0718	−0.3067
CII. SL-crossbreds, transpl.	27	0.0086	−0.4140	−0.4947[1]
DII. LS-crossbreds, transpl.	22	−0.2795	−0.2593	−0.0987
E. Pure breeds born by crossbreds	26	0.1217	0.2013	0.2595

[1] $P < 0.05$; [2] $P < 0.01$.

VOL. 31 1950

and it will generally be permissible to ignore the modifying influence of this factor. This is in disagreement with MINOT (1891), who supposed that the differences in the birth weights of guinea pigs were caused by variations in the length of the gestation period. KEILMANN (1891) advanced the theory that it is the uterine pressure which starts the parturition, and that when the muscular tension in the uterus which acts on the ganglions in *Pars supravaginalis* has reached a certain level the parturition commences. This conclusion is in fact in good agreement with the negative correlations between the size of the litter and the length of the gestation period, for the length of the gestation period would consequently be inversely proportional to the size of the litter. It does not explain, however, the positive relationship between the length of the gestation period and the litter weight, for if KEILMANN's hypothesis was correct, this correlation should be negative, as the litter size is proportional to the litter weight. A positive correlation between the length of the gestation period and the birth weight was likewise found by KOPEĆ (1923) and WISHART and HAMMOND (1933) for rabbits, by WRIGHT (1921) and EATON (1932) for guinea pigs and by other workers. It is therefore far more probable that the length of the gestation period is determined by interacting factors, and in addition to the purely physical effect there is at the same time a physiological factor which plays a far more important part than has been generally supposed. If the pressure of the fetuses, whether of the individual fetus or of the whole litter, was of decisive importance, this would be clearly shown by considerable differences in the length of the gestation period in the reciprocal transplantations. In both cases of the pure breeds, positive, though small, correlations have been found between the length of the gestation period and the litter weight. The mean gestation period is shorter for Large race borne by Polish (see

Table 10) than for Polish borne by Large race does, but the former mean gestation period corresponds to a mean litter weight of 170.2 gms., whereas the mean gestation period for Polish borne by Large race does, which is one day longer, corresponds to a mean litter weight of 195.3 gms.

The partial correlations between maternal weight and litter weight in the two experimental groups covering the pure breeds call for further explanation. In one group, Large race borne by Polish, the correlation is in complete agreement with the correlation for normal Large race (Table 21), but in the other group, viz. Polish fetuses developed in Large race does, the coefficient is negative and too large to be ignored. In the cases where the mothers have been Flemish Giant does, the litter weight or the average weight per young have been less than in those where the mothers have been crosses between Flemish Giant and Vienna Blue or pure Vienna Blue, although the mean weight of pure Flemish Giant mothers is higher than of the others. It is therefore, perhaps preferable to calculate the correlation between maternal weight and litter weight within breeds. If this is done the partial coefficient between the maternal weight and the litter weight within litter size within breeds falls to —0.2117, and consequently there is no great difference between this and the former coefficient. Neither of the coefficients is significant.

A further examination of the individual data leads to the conclusion that the whole of the difference between Flemish Giant mothers on the one hand and Vienna Blue and Large race crosses on the other in their effects on the litter weight, can be traced back to a couple of individuals purchased from outside, and which proved to be very poor mothers. It does not seem possible to find a satisfactory biological explanation of the observed negative correlations between the maternal weight and the litter weight, and as none of the correlations found in this case can be regarded as significant, it would appear to be justifiable to interpret these results as being due to chance.

For the crossbreds between Large race and Polish it has only been possible to calculate the correlations for the transplanted material. Here it appears that the partial correlations between the length of the gestation period and the litter weight are negative within both groups, while the corresponding coefficients for the two pure breeds are positive. The mean length of the gestation period for SL-crossbreds borne by Polish does is 31.80 days, to which corresponds an average litter weight of 147.6 gms., whereas for LS-crossbreds borne by Large race does it is 32.39 days with an average litter weight of 224.9 gms. If these figures are compared with those for the pure breeds, it would appear that the size of the mother has a more decisive influence on the length of the gestation period than has the genetical composition of the fetuses. It is not possible to enter into a detailed examination of the differences in the length of the gestation period between pure breeds on the basis of the present experimental data, for the transplantations take place by means of surgical

operations which may disturb the normal behavior of the Fallopian tubes. Amongst other factors, there may be a temporary delaying of the passage of the ova through the Fallopian tubes, in some way dependent upon how near the ova are deposited to the fimbrial end of the tube, and likewise dependent upon the quantity of fluid injected at the implantation of the ova. Moreover, it appears most likely that the peristaltic movements in the Fallopian tubes are considerably slowed down on account of the anaesthesia, and finally it is quite conceivable that the development of the ova is checked during or after the storage *in vitro*, so causing delayed implantation. On the average, the developmental period of transplanted fetuses is longer than that of the normal pure breeds. Further, it cannot be decided on the basis of this data whether there is an intermediate length of gestation for crosses between the two pure breeds, as has been shown for crosses between certain cattle breeds (Long *et al.* 1948).

The correlation between maternal weight and litter weight for the SL-crossbreds is positive but insignificant, whence it must be concluded that there is no relation between the two factors when the litter size and the gestation period are kept constant. The coefficient found is entirely in agreement with that for normal Polish within litter size ($_{w}r_{12 \cdot 3}$), but it disagrees with the partial correlations between maternal weight and litter weight for Large race borne by Polish. Consequently it is considered probable that within certain limits the litter weight is independent of the size of the mother, but if the litter weight exceeds a certain value, as is the case of Large race developed in Polish does, it is affected not only by intra-uterine space, but also by abdominal size. In other words, the total capacity of the abdomen and the compactness with which the abdominal organs are placed in relation to each other exert an indirect influence on the developmental potentialities of the fetuses. In this case it is a question of modifying agents of a purely physical as well as of a purely physiological nature.

In the other cross group, LS-crossbreds borne by Large race, a parallel is found to the correlation between maternal weight and litter weight, as was found in Polish borne by Large race. Both coefficients are negative. However, the positive correlation for normal Large race is in disagreement with these results. With regard to the larger litters there are probably, in the latter case, physical and physiological conditions which influence the litter weight, while the purely physical size differences are without importance for the embryonic development in mothers which are one and a half to four times as large as the natural mothers of the fetuses.

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In order to ascertain the heritability of a character or a group of characters, it is, as newly has been strongly emphasized by Lush (1949), extremely important to know the influence of the environmental factors. Even though the

VOL. 31 1950

subject of the present investigations is not direct inheritance of size, it is nevertheless important to know which factors must be taken into account and which exert so slight an influence that they can be left out of consideration without, however, introducing errors which would interfere with the interpretation of the results. A number of these factors have been examined, and the conclusion is reached that by far the most important factor influencing birth weight (litter weight) is the size of the litter, which is also significantly correlated with the gestation period (in the same way as is the litter weight). On the other hand, no intra-breed correlation between the size of the mother and the size of the litter has been found in other cases than in normal Polish. With constant litter size, most of the correlations are reduced considerably, and significance is only to be found in a few groups. Therefore the length of gestation will not be considered in the calculations in the following sections. Similarly, the seasonal influence (in no case has seasonal variation been ascertained), the influence of the age of the mother, the litter number and the sex, and the difference between living and stillborn young will be left out of consideration. However, as significant correlations between the weight of the mother and the weight of the litter have been found in several groups, this fact will be considered subsequently in the calculation of the relative birth weight in all cases where the weight of the mother at the time of mating is known.

ANALYSES OF VARIATION IN THE BIRTH WEIGHT.

A. *Large race.*

Pure Flemish Giant, pure Vienna Blue, and crosses between them have been used as Large race. Prior to 1946 Large Chinchilla was crossed into the stocks, but since that year the combinations mentioned first have been used exclusively. The objection may be made that heterogeneity which is difficult to control has thereby been introduced, and further that the crossing of pure races may cause heterosis. However, it was assumed *a priori* that any possible heterosis effect would only manifest itself through greater viability and a stronger constitution and that it would not cause any great difference in the birth weight of the young of the different race groups. The experiments have shown the validity of this hypothesis. The tables below comprise analyses of the three "races", in which Flemish Giant have been treated as one group, Vienna Blue as another, and crosses between the pure large breeds have been collected into a third group. The data has been examined firstly with regard to differences between the does, and significant differences between the three race groups have been found (Table 22). The mean weight of Flemish Giant was 4.94 ± 0.081 kgms., of Vienna Blue, about one kgm. less, 3.93 ± 0.053 kgms., while the mean weight of Large race crosses was 4.10 ± 0.055 kgms. These figures are for all indi-

TABLE 22. Differences between adult weight for Flemish Giant, Vienna Blue, and Large race crosses as donors, receptors, and mothers of normal litters.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance, %
Total	192	0.4447		
Between breeds	2	18.3479	28.65[3]	51.21
Between donors, receptors and mothers	6	0.6404	2.63[1]	3.44
Within donors, receptors and mothers	184	0.2437		45.35

[1] $P < 0.05$; [3] $P < 0.001$.

viduals used, whether they have borne litters or been parents of offspring as donors. From this it is clearly seen that the whole difference is between Flemish Giant on the one hand and Vienna Blue and crosses on the other. If the material is reduced to include only those donors which are parents of offspring, the mean weight for Flemish Giant is 4.65 ± 0.074 kgms., and for Vienna Blue together with crosses, 4.00 ± 0.057 kgms. The difference is significant ($P < 0.001$). The slight influence which Flemish Giant has had on the material is due to the fact that only 18.6 per cent of the 43 donors used were of this breed, so that the mean for all donors is 4.12 ± 0.074 kgms., which is but little above the figures for the other two large breeds, in spite of the much greater weight of Flemish Giant. Similarly, the young of Flemish Giant borne by Polish represent 20.5 per cent of the 78 young which comprise this group. Further, it is seen from Table 22 that there is a slight significant difference between the mean weights of donors, receptors, and mothers of normal litters, but the difference is without any importance, for only about 3.5 per cent of the total variation in maternal weight for Large race is between these groups, whereas 50 per cent of the variance must be ascribed to differences between the various race groups. Some of the variance between donors, receptors, and nor-

TABLE 23. Differences between birth weight of normal Flemish Giant, Vienna Blue, and Large race crosses.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance, %
Total	547	181.83		
Between litter sizes	11	1764.05	2.07	10.81
Between breeds	15	851.91	1.06	1.36
Between litters	39	800.88	10.69[3]	47.33
Within litters	481	74.94		40.50

[3] $P < 0.001$.

VOL. 31 1950

mal mothers is partially attributable to a difference between years, and partially due to age differences between the animals. The weights for all donors and receptors were recorded on the day when the animals were killed or operated on, i.e. in most cases at a comparatively early age, whereas the figures for normal mothers represent the weights of the animals when fully grown, i.e. the mean weights at the ages of nine to ten, eleven, and twelve months according to breeds.

What is, however, of major importance in these investigations is to find the relationship between the birth weight of young developed under normal conditions and that of young developed in an alien uterine environment. It is important that no difference exists between the birth weights of young in the three different large breeds, as has been shown in Table 23, for this permits the pooling of the data. The part of the variance that can be ascribed to differences between the groups of Large race does not represent more than 1.36 per cent of the total variance, or about one thirtieth of the variance within litters. It must therefore be considered legitimate in this material to regard these various fetus groups as belonging to one "race" as far as the birth weight is concerned. For smaller breeds on the other hand, it is probable that the same proportionate difference in the maternal weight will cause significant differences in the birth weights. This was shown by Kopeć (1923, 1924) for Himalaya and Small Silver, of which the heavier race weighs about 25 per cent more than the lighter. The same difference is found between Flemish Giant on the one hand and Vienna Blue and crosses on the other.

A1. Normal litters.

For the normal material information on the birth weight of 547 out of 550 young in 66 litters is available. As may be seen in Table 24, the litter size has varied from only one to thirteen young. The average litter size is 8.33 ± 0.34.

Table 23 further shows the proportion of the variation due to the litter size. It is rather surprising that the modifying influence on the birth weight represents only 10.81 per cent of the calculated total variance of 185.06, but this is entirely attributable to the very uneven distribution of the litters between the different litter sizes. Incidentally, the difference between the mean birth weights for litter sizes is not significant. From Table 24, it can be calculated that about 6 per cent of the 66 observed litters belong to litter sizes of four or less, 26 per cent to the litter sizes of five to seven, 47 per cent to the group of eight to ten young in the litter, and 21 per cent to the group of eleven to thirteen. By far the greater part of the total variance is between litters, although within litters there is also very great variation, and the variance "within litters within litter sizes" amounts to 40 per cent of the total.

It is obvious that with such great variations within litter sizes it is difficult

TABLE 24. Number of young and mean birth weight of Large race fetuses.

Litter size	Normal			Transplanted		
	Number of young	Mean birth weight, gms.	Estimated mean birth weight, gms.	Number of young	Mean birth weight, gms.	Estimated mean birth weight, gms.
1	2	109.0	102.2	6	77.8	91.4
2	—	—	86.3	14	70.4	68.3
3	3	93.3	78.1	6	65.3	57.7
4	4	70.0	72.8	8	55.5	51.1
5	35	74.5	68.9	20	47.9	46.5
6	29	60.1	65.8	24	42.1	43.1
7	34	63.3	63.4			
8	87	60.1	61.3			
9	88	61.5	59.6			
10	100	61.2	58.1			
11	66	62.1	56.7			
12	60	55.1	55.5			
13	39	53.0	54.4			
Total and goodness of fit.	547		0.3316[a]	78		0.8272[a]

[a] $P < 0.001$.

to find a function which is in complete agreement with the observed values. However, it appears that the correlation between the observed values and those calculated by the three functions previously discussed is highly significant ($P < 0.001$). The values for the power function are shown in Table 24. The best agreement between the observed and the calculated figures is for $y = 102.24 \cdot x^{-0.2464}$, ($R = 0.3316$). In accordance with YULE and KENDALL (1948) the symbol R is used for these correlation coefficients. The straight line $y = 79.25 - 1.95\,x$ is likewise in good agreement with the observed means ($R = 0.3313$) if the smallest litter sizes are ignored, while the exponential function, $y = 79.02 \cdot 1.031^{-x}$, shows worse fit ($R = 0.3085$). There is no great difference between the functions. It is seen from the table that the birth weights of the singly born young are very high. In the two litters of only one young the weights have been 116 and 102 gms. for Flemish Giant and Vienna Blue respectively. With an increasing number of young in the litter, the weights fall rapidly, and when the number of young in the litter exceeds six or seven it is very rare to find birth weights above 65 gms. Owing to the uneven distribution of the litters between litter sizes it is questionable whether

VOL. 31 1950

in fact it would not be more correct to calculate the function on the basis of the observed means. If this is done the power function is $y = 107.15 \cdot x^{-0.2568}$ with a correlation between observed and calculated values of 0.7944 ($P < 0.01$).

A II. Transplanted litters.

The material which covers the young of Large race borne by Polish, and for which the birth weights are known, comprises 78 young in 25 litters (Table 24). The mean litter size is only 3.12 ± 0.38 young, or not half as large as in the normal material. Likewise the litter size has varied less, no litters of more than six having been observed. The mean weight of the receptors is 1.49 ± 0.056 kgms., whereas the mean weight of the Large race donors which have been parents of young in this group is 4.16 ± 0.087 kgms., which does not differ from the mean weight of all donors used.

The average birth weight for each litter size of young of Large race borne by Polish is shown in Table 24. The birth weight of singly born young is about 80 gms., the highest weight observed being 90 gms., and the lowest 70. With an increasing number of young in the litter the birth weight per young decreases greatly, and in a litter size of six the average birth weight is only about one half of the weight of singly born young. When the litter size has such a large modifying influence on the birth weight it is evident that a very great part of the variation found must be due to differences between the different litter sizes, and the analysis of variance (Table 25) shows that 69.13 per cent of the total variance must be attributed to this source of variation. Only 13.98 per cent of the variance is due to differences between litters of the same size, while the remainder falls within litters and is presumably caused principally by differences between the sexes and by various genetic factors.

The birth weight of the individual young follows fairly closely the power function $y = 91.42 \cdot x^{-0.4197}$ with a correlation, R, between the theoretical and the observed values of 0.8272 ($P < 0.001$). The standard deviation is $(\overset{\times}{:})$ 1.1635. (The standard deviation for the regression line is calculated as $\pm$ log., which transformed to anti-log. gives multiplied or divided by, $\overset{\times}{:}$). However, the

TABLE 25. Analysis of variance of birth weight of transplanted Large race fetuses.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance, %
Total	77	223.37		
Between litter sizes	5	2411.73	15.86[3]	69.13
Between litters	19	152.11	3.58[3]	13.98
Within litters	53	42.46		16.89

[3] $P < 0.001$.

exponential function is also in good agreement with the observed values ($R = 0.8249$, $P < 0.001$), as has already been shown on page 64 for the straight line within the range of observed litter sizes, ($R = 0.8355$, $P < 0.001$). Calculated on the means the power function is $y = 84.38 \cdot x^{-0.3342}$ with $R = 0.9381$ ($P < 0.01$). The values calculated from the power function are entered in Table 24.

B. *Small race.*

The small race individuals used have all been Polish rabbits of the albino type, i.e. white with pink eyes (recessive), with the exception of two does that were white with blue eyes (dominant). All the animals have been homozygous with regard to the colour, whereupon it has been possible to distinguish with absolute certainty transplanted offspring from normal offspring immediately at birth. In the normally performed transplantations, albino offspring have not been observed in any case when fertilized ova from Large race have been transplanted to Polish. The appearance of Polish young in the same litter as transplanted offspring of Large race will be dealt with in a later section, but it can be stated here that it was a case of ova transplanted to a normally mated doe and not a case of parthenogenetic offspring, as found by PINCUS (1939).

VOL. 31 1950

B_1. Normal litters.

The mean weight of 150 mothers of normal litters is 1.62 ± 0.030 kgms. at the age of nine months, at which age Polish must be considered full-grown. Unfortunately the population has not been as homogeneous as might be desired, due largely to the fact that it has been necessary to buy fresh individuals in order to maintain the population. Further, a possible post-natal influence after development of Polish fetuses in Large race does may have contributed to make some of the animals larger than the normal for the race. Information about the birth weight is available for 161 litters comprising 687 young. Nine different litter sizes are represented, the average being 4.46 ± 0.14.

TABLE 26. Analysis of variance of birth weight of normal Polish fetuses.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance, %
Total	686	66.15		
Between litter sizes	8	643.90	4.02[a]	9.46
Between litters	152	160.17	5.31[a]	45.47
Within litters	526	30.19		45.07

[a] $P < 0.001$.

TABLE 27. Number of young and mean birth weight of Polish fetuses.

Litter size	Normal			Transplanted		
	Number of young	Mean birth weight, gms.	Estimated mean birth weight, gms.	Number of young	Mean birth weight, gms.	Estimated mean birth weight, gms.
1	5	52.6	50.5	1	56.0	61.3
2	37	43.5	45.3	16	57.8	59.1
3	78	45.0	42.5	6	62.3	57.9
4	119	41.4	40.7	16	62.2	57.0
5	145	39.7	39.3	5	53.8	56.4
6	172	38.8	38.2	12	56.4	55.9
7	72	40.2	37.3	—	—	55.4
8	50	35.2	36.5	8	51.9	55.0
9	9	33.3	35.8			
Total and goodness of fit.	687		0.2845[3]	64		0.1556

[3] $P < 0.001$.

An examination of the causes of variation shows that in this case too the litter size is of minor importance in the modification of the birth weight, even though the difference between litter sizes is highly significant, as shown in Table 26, ($P < 0.001$). The variance amounts to only 9.46 per cent of a total variance of 66.99. The remaining part is fairly evenly distributed within litter sizes, with 45.47 per cent between and 45.07 per cent within litters.

For normal Polish young there is in general comparatively good agreement between the value found for the birth weight of different litter sizes and those calculated from the three different functions. That, in spite of the high significance ($P < 0.001$), the coefficients of correlation are not higher is due to the great variation within litter sizes, as for Large race. The best agreement between observed and calculated values is shown by the linear equation $y = 47.81 - 1.46\,x$; ($R = 0.2968$, $P < 0.001$), which, however, as pointed out previously, must be regarded as unreasonable. Of the two other functions which have been examined, $y = 50.48 \cdot x^{-0.1560}$ is found to be the better fit ($R = 0.2845$, $P < 0.001$), but there is no great difference between this and the exponential function $y = 47.38 \cdot 1.036^{-x}$; ($R = 0.2822$, $P < 0.001$). The standard deviation is $\binom{x}{\cdot}$ 1.1701 for the first function and $\binom{x}{\cdot}$ 1.2097 for the second.

The observed mean weights for the different litter sizes together with the calculated figures from the power function are shown in Table 27, from which it appears that the birth weights of the singly born young are about 52—53 gms.,

TABLE 28. Analysis of variance of birth weight of transplanted Polish fetuses.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance, %
Total	63	98.76		
Between litter sizes	6	136.10	—	(negative)
Between litters	12	298.29	7.35[3]	65.34
Within litters	45	40.58		34.66

[3] $P < 0.001$.

the weight decreases with an increasing number of young in the litter, and the average birth weight of individuals in the largest litters is about 33—35 gms. If the birth weights is calculated on the basis of the means, the power function is, $y = 52.28 \cdot x^{-0.1751}$; ($R = 0.9397$, $P < 0.001$).

VOL. 31 1950

B II. Transplanted litters.

In the experimental group comprising transplantations of fertilized ova from Polish to Large race does, pregnancy has been brougth about in 19 cases, and a total of 64 young in seven different litter sizes have been born. Thus the average litter size is 3.37 ± 0.42 young, which is approximately one less per litter than in the normal material, in spite of the fact that the mothers of the experimental litters have belonged to Large race. The litter size has varied from one to eight. The mean weight of the receptors is 4.17 ± 0.205 kgms., while the donors which have been parents of offspring have an average weight of 1.56 ± 0.051 kgms.

The average birth weight of Polish young borne by Large race does is seen in Table 27. The weights are distributed very irregularly and no definite trend can be ascertained. There is only one litter of size one, so no great importance can be attached to this figure, but litter size two shows a lower mean than litter size four (16 young in each), and litter size five has a lower mean than litter size six. The mean birth weight of all the young is 58.0 gms., and the analysis of variance shows no statistically significant deviation from this mean for any of the litter sizes (Table 28). The whole of the variation is within litter sizes, two thirds being between and one third within litters. The total "computed variance" amounts to 117.09. By examining the data for the individual litters it was found that the highest birth weight observed was that of an individual born in a litter of four. The average weight in this litter was 78 gms. and the heaviest young weighed 86 gms. The smallest in this group, likewise born within litter size four, weighed 40 gms.

An examination of the functions reveals a very poor fit with the observed

values. The straight line equation, $y = 61.45 - 1.05\,x$; ($R = 0.2120$), and the exponential function, $y = 61.73 \cdot 1.018^{-x}$; ($R = 0.2132$), are slightly better fits than the power function, $y = 61.27 \cdot x^{-0.0516}$; ($R = 0.1556$), but the correlation is not significant in any case. There is accordingly complete agreement between the functions examined, and the analysis of variance in which no significant effect of litter size could be shown. The weights calculated from the power function are shown in Table 27. The only plausible explanation which can be given is that the intra-uterine environment provides such favourable conditions for development of the small fetuses (Small race), both with regard to space and nutrition, that the litter size does not act as size-modifying factor. It is therefore justifiable to draw the conclusion that the birth weights observed do in fact represent the maximum development of the Polish fetuses which the genetic constitution would permit under any environmental conditions. A small part of the variation in the birth weight between litters must be attributed to physiological differences between the foster-mothers, but these cannot be regarded as having any great effect. Part of the variation is likely to be due to errors (in some cases the young have not been weighed immediately they were born), but by far the greater part is probably caused by differences in genetic constitution between litters or fetuses of different origin.

C. *Crossbreds from Polish male and Large race female.*

The greatest part of the material comprising normal crossbreds between Small race ♂ and Large race ♀ consists of crosses between Polish and Flemish Giant, only one litter being the result of a cross between Polish and Vienna Blue. Since 1946 normal matings have taken place in very few cases, but no difference in birth weight and adult weight between litters born prior to 1946 and the litters from recent years has been detected. In the following, the crosses within this group will be designated as SL-crossbreds.

C_1. Normal SL-crossbreds.

The normal material comprises 14 litters totalling 110 young, in litter sizes from two to thirteen. The smallest litter sizes are represented by only one litter each, while the highest class is litter size nine, represented by three litters or 27 young. The mean litter size is 8.21 ± 0.89. The mean weight of the mothers of the normal litters is 3.92 ± 0.221 kgms., which does not differ from the other groups of Large race.

An examination of the different causes of variation (Table 29) shows that there is a highly significant difference between litter sizes ($P < 0.001$), whereas there is no difference between the litters. However, an examination of the difference between litters will not in this case give a reliable estimate of the

TABLE 20. Analysis of variance of birth weight of normal SL-crossbred fetuses.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance, %
Total	109	86.37		
Between litter sizes	9	417.65	7.38[3]	34.39
Between litters	4	79.56	1.43	3.41
Within litters	96	55.60		62.20

[3] $P < 0.001$; tested on basis of the mean square "within litter sizes" (= 56.56).

importance of this cause of variation both because of the small number of litters and because it is only within the litter sizes nine, ten, and thirteen that more than one litter were observed. Therefore calculations must be restricted to an estimate of the difference between the means for the few litters represented by these three litter sizes. In a partition of the variance, which is 89.39 for the whole group, 3.41 per cent is accounted for by the difference between litters, and 34.39 per cent must be attributed to the difference between litter sizes. The remainder, 55.60 or 62.20 per cent of the total variance, represents principally the variance within litters.

On the whole, the functions examined show good agreement between observed and calculated data. Thus the correlation is 0.5254 ($P < 0.001$) for the power function $y = 86.02 \cdot x^{-0.2625}$ with a standard deviation of ($\frac{x}{.}$) 1.1734. As with the groups discussed previously, the straight line, $y = 65.48 - 1.72\,x$, shows quite good agreement within the given interval ($R = 0.5066$, $P < 0.001$). The values calculated from the power function have been compiled in Table 30 together with the observed weights. It is seen that the weight per fetus at birth in a litter size of two is 76.5 gms., whereafter the birth weight decreases rapidly in the succeeding litter sizes, and in litters of more than nine young it is about 45 gms. With an observed weight of 76.5 gms. for twins it is most likely that the initial weight, i.e. the mean weight of singly born young, for crosses between Small race buck and Large race doe is about 85 gms. The power function shows a similar initial weight, while the semi-logarithmic function, $y = 66.44 \cdot 1.034^{-x}$, gives a weight of 64.3 gms. for singly born young. The correlation for this function between observed and calculated values is a little less marked than for the former two, $R = 0.4889$ ($P < 0.001$), but still highly significant. Calculated on the means for each litter size the power function, $y = 88.21 \cdot x^{-0.2684}$; ($R = 0.9427$, $P < 0.001$) is found. The variation within litter sizes is thereby entirely eliminated, but at the same time an uncertainty arises since for each of the means used there is an error.

VOL. 31 1950

C_{II}. Transplanted litters.

In this experimental group a total of 73 young have been born in 27 litters, ranging in size from one to seven with the exception of litter size five. The average litter size is 2.70 ± 0.30 young. As previously mentioned the experiments have been carried out so that fertilized ova of a cross between Polish male and Large race female have been transplanted back to the breed of the sire, in this case Polish. Therefore the litter size will be compared with the mean litter size of normal Polish and that of Large race borne by Polish. The litter size has been smaller in this group than in the two other groups. The mean weight of the receptors, 1.58 ± 0.043 kgms., does not differ significantly from that of the other groups. The weight of the donors, which have been parents of offspring in this group, is 4.09 ± 0.145 kgms., while the males weighed 1.54 ± 0.070 kgms.

The average birth weight is shown in Table 30. The initial weight (litter size one) is 70 gms., but with a larger number of yong in the litter the weight decreases considerably. In litter size four the mean birth weight is about 50 gms., while for litter sizes of more than six the weight falls below 40 gms.

TABLE 30. Number of young and mean birth weight of SL-crossbred fetuses.

Litter size	Normal			Transplanted		
	Number of young	Mean birth weight, gms.	Estimated mean birth weight, gms.	Number of young	Mean birth weight, gms.	Estimated mean birth weight, gms.
1	—	—	86.0	7	69.7	75.2
2	2	76.5	71.7	14	61.9	61.8
3	3	60.3	64.5	15	58.7	54.1
4	—	—	59.8	24	51.4	49.6
5	5	57.0	56.4	—	—	46.4
6	6	59.3	53.7	6	42.8	44.0
7	7	52.3	51.6	7	37.1	42.0
8	8	51.0	49.8			
9	27	45.7	48.3			
10	19	50.1	47.0			
11	7	43.6	45.8			
12	—	—	44.8			
13	26	45.7	43.8			
Total and goodness of fit.	110		0.5254[3]	73		0.7122[3]

[3] $P < 0.001$.

TABLE 31. Analysis of variance of birth weight of transplanted SL-crossbred fetuses.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance, %
Total	72	141.85		
Between litter sizes	5	1164.18	6.44[3]	51.86
Between litters	21	180.77	13.94[3]	39.82
Within litters	46	12.97		8.32

[3] $P < 0.001$.

The difference between the mean birth weights in the different litter sizes is highly significant ($P < 0.001$), just as is the difference between litters within litter sizes ($P < 0.001$). The analysis of variance (Table 31) shows that 51.86 per cent of the total "computed variance" of 155.86 is due to differences between litter sizes, while approximately 40 per cent is between litters. It is remarkable that the variance within litters amounts to only one twelvth of the total variance. By way of comparison it might be recalled that the corresponding part of the variance for transplanted Large race was one sixth. The absolute variance is in fact considerably smaller for crossbreds than for Large race.

From the data the power function $y = 75.19 \cdot x^{-0.2996}$ is calculated with $R = 0.7122$ ($P < 0.001$), and the exponential function is $y = 76.24 \cdot 1.105^{-x}$ with $R = 0.7585$ ($P < 0.001$). The best agreement between observed and calculated values is consequently to be found for the "semi-logarithmic" function, in which it is assumed that the decrease in weight per young is proportional to the increase in the number of young in the litter. The standard deviation for the first curvilinear regression is $\left(\frac{+}{-}\right)$ 1.1715 and for the second, $\left(\frac{+}{-}\right)$ 1.1584. Good agreement between the values is also to be found for the straight line $y = 71.88 - 4.93\,x$; ($R = 0.7420$, $P < 0.001$). It is true of each of the two latter cases that the functions decrease very quickly, so that in a litter size of twelve, which is quite possible, the weight per young would be so low that it is improbable that viable offspring would be born. The question whether or not the development of the fetuses follows one of the functions for a very large number of young in the litter cannot be answered by these studies, but it is mentioned in passing that this problem will be taken up for direct investigation in later experiments. Calculated on the means, the logarithmic function is found as $\log y = 1.8749 - 0.3106 \cdot \log x$ or $y = 74.97 \cdot x^{-0.3106}$; ($R = 0.9496$, $P < 0.01$).

D. *Crossbreds from Large race male and Polish female.*

As in the last group, most of the crosses in this group are, so far as the normal material is concerned, between Flemish Giant and Polish, and the

VOL
31
195

majority of the litters entering into the calculations were born before the transplantation experiments were started. Since 1946, normal matings of Vienna Blue buck with Polish doe have resulted in five litters. Besides, the material for normal has been supplemented artificially by three small litters. This experiment will be discussed in due course. In the following, all the crosses in this group will be designated LS-crossbreds.

D_1. Normal LS-crossbreds.

The normal material covers 35 litters with 128 young, born in litter sizes of one to seven, but the distribution is such that 110 young, or 86 per cent, have been born within the litter sizes three to five. This fact makes comparisons between normal and transplanted litters outside this interval very precarious. Calculated on the above data, the mean litter size is 3.77 ± 0.21. The average weight of the mothers of the normal litters is 1.57 ± 0.084 kgms.

Litter size one is represented in the normal material by only one young whose weight was 32 gms., i.e. considerably below the mean weight for all the other litter sizes. It is very seldom that this happens in rabbits, but as mentioned on page 63 it is a fairly common phenomenon in other multiparous animals. It was observed by HAMMOND (1921) for rabbits, but for all the material covered by these investigations it has not been observed in any of the other normal groups. The question naturally arises of what may be the cause of the strongly deviating birth weight for singly born young. From the experience and results of these investigations it can be asserted that there is nothing unnatural in the low birth weight, but it would be wrong to regard such a young as belonging to litter size one. It is far more probable that the actual litter size was eight or ten, or more, but because of a very high uterine mortality only the most vigorous fetus survived to be born alive. The others would be resorbed during the gestation period. No definite proof of this hypothesis can be given, but it is strongly supported by data for the few supplementary litters added to this group. In eight cases one to three fertilized ova from Polish doe mated with Large race buck have been transplanted to Polish doe. Unfortunately only three does became pregnant, due to the fact that the constitution of the animals was impaired by disease. In one litter one young was born, which weighed 64 gms., i.e. its weight was twice as high as the weight previously found for a singly born young. The two other litters consisted of two young each, the weights of which were 60 gms. and 60 gms., and 62 gms. and 59 gms. These weights are, as was to be expected, a little below the weight of the singly born young. It must be admitted that the data is inadequate for the drawing of any final conclusions, and for this reason these supplementary litters will simply be added to the rest of the material, which will thus be increased to 133 young in 38 litters. In order to increase

TABLE 32. Analysis of variance of birth weight of normal LS-crossbred fetuses.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance, %
Total	132	49.79		
Between litter sizes	6	165.74	1.74	7.34
Between litters	31	95.02	3.43[3]	37.96
Within litters	95	27.71		54.70

[3] $P < 0.001$.

further the number of individuals in the small litter sizes, several attempts have been made to mate the does to vasectomized bucks and again, after seven or eight hours, to fertile bucks (HAMMOND 1934). After repeated unsuccessful attempts the idea was abandoned.

The partition of the total variation into that attributable to the various causes is shown in Table 32. There is no significant difference between litter sizes, probably because of the distribution of the litters. On the other hand, the difference between litters is highly significant ($P < 0.001$), even if 54.70 per cent of the total "computed variance" is to be found within litters. Approximately 38 per cent of the variance is between litters.

With such great variation within litters it is obvious that a mathematical function can be of only approximate validity. Nevertheless there is satisfactory (significant) agreement between the observed and the calculated values for the three functions examined. The logaritmic function, $\log y = 1.7341 - -\log x \cdot 0.1088$ or $y = 54.21 \cdot x^{-0.1088}$, shows slightly better agreement ($R = 0.2493$, $P < 0.01$) than the semi-logarithmic function, $\log y = 1.7212 - -0.0125\,x$ or $y = 52.62 \cdot 1.029^{-x}$; ($R = 0.2399$, $P < 0.01$). The standard deviation for the former is $\binom{x}{y}$ 1.1578 and for the latter $\binom{x}{y}$ 1.1582. In this case also the straight line is best fit with the observed values. The equation for this is $y = 54.68 - 1.45\,x$; ($R = 0.2591$, $P < 0.01$). If the equation is based on the means for each litter size, and if the fetus weight of 32 gms. is omitted from the calculations, there is excellent agreement between the observed and the calculated values. This equation is $y = 62.42 \cdot x^{-0.1786}$; ($R = 0.9288$, $P < 0.01$). Observed mean weights and estimated weights from the power function are shown in Table 33. No great importance can be attached to the observed initial weight of 48 gms., as the weight of singly born young should probably be over 60 gms. For larger numbers of young in the litter a normal decrease in the average weight sets in, until in litter size 7 it is only 45 gms. Between litter sizes 1 and 7 the decrease represents 25 per cent, which is in full agreement with the results for normal Polish.

VOL
31
195

D_{II}. Transplanted litters.

This experimental group deals with the transplantation of fertilized ova from Polish doe mated with Large race buck to Large race does. The material comprises 78 young in 22 litters, varying in size from one to seven, i.e. the same as in the normal material. The average litter size is 3.55 ± 0.31, or slightly greater than for Polish borne by Large race does. The mean weight of the receptors is 4.04 ± 0.099 kgms., and that of the donors, 1.64 ± 0.056 kgms. The bucks used to donors have a mean weight of 3.92 ± 0.146 kgms. Thus none of the animals used differ in size from those used in the other groups.

The observed means for crossbred young of Large race male by Polish female, borne by Large race does, have been compiled in Table 33, from which it is seen that the initial weight is 92 gms., the weight of litter size two is 18 gms. less, while the decrease in weight with a further increase in the number of young is only about 8 gms. With six or seven young in the litter the weight per young is about 56 gms., which is very much the same as was found in Polish borne by Large race does (cf. Table 27). On the other hand, the initial weight of the crossbreds in no way corresponds with the initial weight for normal Large race, which latter is much higher, nor does it correspond with the initial weight of Polish borne by Large race. In the latter case there was no difference in the birth weight whether the litters were small or large. The weights appear to correspond rather more to those for normal SL-crossbreds. An examination of the different causes of variation (Table 34), shows a highly

TABLE 33. Number of young and mean birth weight of LS-crossbred fetuses.

Litter size	Normal			Transplanted		
	Number of young	Mean birth weight, gms.	Estimated mean birth weight, gms.	Number of young	Mean birth weight, gms.	Estimated mean birth weight, gms.
1	2	48.0	54.2	3	91.7	86.3
2	9	56.6	50.3	2	74.0	73.5
3	35	48.0	48.1	30	66.3	66.9
4	36	46.7	46.6	4	61.1	62.5
5	39	45.4	45.5	20	61.4	59.4
6	5	48.8	44.6	12	56.1	56.9
7	7	44.9	43.8	7	55.9	54.9
Total and goodness of fit.	133		0.2493[2]	78		0.6651[3]

[2] $P < 0.01$; [3] $P < 0.001$

TABLE 34. Analysis of variance of birth weight of transplanted LS-crossbred fetuses.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance, %
Total	77	102.48		
Between litter sizes	6	669.61	5.19[2]	44.20
Between litters	15	129.03	3.73[3]	24.26
Within litters	56	34.61		31.54

[2] $P < 0.01$; [3] $P < 0.001$.

significant difference between litter sizes ($P < 0.001$), while the difference between litters is also significant ($P < 0.01$), 44.20 per cent of a total variance of 109.75 is attributable to litter sizes and 31.54 per cent to variation within litters. The remaining 24.26 per cent is accounted for by differences between litters.

The best agreement between observed and calculated values in this material is for $\log y = 1.9361 - \log x \cdot 0.2326$; ($R = 0.6651$, $P < 0.001$), whereas the poorest is for $\log y = 1.9081 - 0.0259 \cdot x$; ($R = 0.5401$, $P < 0.001$). In the former case the standard deviation (log) is ± 0.0737 and in the latter ± 0.1291. In this group too the straight line is in good agreement with the observed means for litter sizes one to seven. The calculated equation is $y = 80.55 - 4.00\,x$; ($R = 0.6199$, $P < 0.001$), and only the initial weight deviates appreciably. In addition to the calculated values for the power (logarithmic) function, $y = 86.32 \cdot x^{-0.2326}$, Table 33 contains the observed means. The function $y = 88.87 \cdot x^{-0.2480}$; ($R = 0.9635$, $P < 0.001$) is based on the observed means of the birth weight.

E. *Pure breeds borne by crossbreds.*

It has been shown that Large race young born by Polish does decrease in birth weight with about 30 to 40 per cent of the normal weight, and that Polish young increase in birth weight by the same percentage after development in Large race does. There is therefore reason to believe that this result would also be found when the two different breeds develop in the same uterus. However, the decrease of birth weight for Large race and the increase for Polish must not be expected to be of the same absolute size. This question has been examined in this experimental series, which covers the simultaneous transplantations of fertilized ova from both of the two pure breeds to does of intermediate size. For receptors, use has been made of both normal crossbreds between the two pure breeds (i.e. crossbred does of Large race × Polish), and reciprocal crossbreds after transplantation. As already mentioned in the Intro-

VOL
31
195

duction, there frequently is a difference in size between these crossbred individuals, and it would therefore be appropriate to examine firstly whether size differences have existed between the groups of mothers. If so, these differences would have to be taken into consideration. Only those mothers have been included which have given birth to litters, and they have been weighed immediately before the transplantation of the ova has taken place, i.e. their weight corresponds to the weight at the time of mating in the other cases. The average weight of all the receptors is 2.98 ± 0.085 kgms. The lightest group of mothers is represented by four crossbred does after Polish ♂ and Vienna Blue ♀, the mean weight of which is 2.61 ± 0.112 kgms. For the two groups of mothers originating from litters derived from reciprocal transplantations the mean weight is 3.05 ± 0.211 kgms. and 3.05 ± 0.100 kgms. No differences are apparent between the mothers ($P < 0.2$), and it is permissible to regard them as belonging to the same population.

The experiments have been carried out so that three ova from Polish have been transplanted to the left Fallopian tube and three from Large race to the right tube. However, by "unilateral" transplantation an entirely identical uterine environment for the two breeds is not provided, unless it is assumed that there is no difference in the potentialities for development in the right or the left side of the uterus. Further, it will not be possible to determine whether the fetuses influence each other positively or negatively (KOPEĆ 1922, 1923 b). However, the problem can be elucidated if ova from both breeds are transplanted to the same uterus horn, so that the ova have, in several cases, been "mixed". Therefore if, for example two ova of Large race plus one of Polish have been transplanted to the one side, one of Large race plus two of Polish have been transplanted to the other side, or the same number of ova of each breed may have been transplanted to both sides. At the same time, in order to restrict to some extent the modifying influence of the litter size, the number of ova transplanted have usually been limited to three of each breed. In only two cases have four ova of one breed been transplanted together with three or four of the other. This method has limited the litter size, and the largest number of young born in a litter was six, comprising in one case four Large race and two Polish, and in another, three young of each.

Pregnancy has been observed in 28 cases in all, but details of the birth weight for two litters are lacking, for which reason the calculations will involve only 26 litters comprising 75 young, of which 40 are Large race and 35 are Polish. A case of cannibalism is the cause of the missing information for one of the two litters, while for the other, the mother died of snuffles 23 days after the operation. An autopsy showed four macerated fetuses, two in each uterine horn. The appearance of the mass indicated that the death of the fetuses had occurred some days previously, and somewhat earlier in the right uterus horn than in the left. Because of the advanced maceration it was un-

fortunately impossible to separate the fetus from the placenta. A weighing of the macerated lumps showed that each one of the two in the left horn weighed 2.02 gms., while those in the right horn weighed 2.22 and 2.17 gms., i.e. both heavier than the fetuses in the left horn, in spite of the fact that, as far as could be judged, the maceration was more advanced. Probably 0.05 to 0.10 gms. should be added to the last of the above figures, as during the removal of the fetus from the uterus a small quantity of fluid was lost. These weight differences are interesting as two ova of Polish had been transplanted to the left Fallopian tube and two of Large race to the right. The observation is consequently in full agreement with the investigations which have shown that the differentiation sets in very early (GREGORY and CASTLE 1931). Further, however, it indicates that the larger fetuses have less power of resistance (whether this be racially caused or not) than the smaller fetuses to pathological conditions in the mother.

In the examination of the different causes of variation it is possible in this group to segregate both the variation which is due to differences in litter sizes and that between the litters, so leaving a part which is attributable solely to differences between and within breeds. The composition of the variance for this material will be taken up for discussion in a later section. The analysis of variance is shown in Table 35. Only a very small part of the whole variation is caused by differences in litter size, and as in the case of the difference between litters, it is completely overshadowed by the variation due to racial differences. The method of analysis which is used here does not show any variance for "between litter sizes" and "between litters" (except a "negative variance"). 90 per cent of the calculated variance of 193.94 is accounted for by racial differences, whereas only 10 per cent can be attributed to differences between litter mates within the same race. If, on the other hand, the variance is exclusively distributed between litter sizes and litters, 4.65 per cent can be attributed to the first and 18.07 per cent to the second cause of variation

TABLE 35. Analysis of variance of birth weight of Large and Small race fetuses borne by crossbred does.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	74	141.48		193.94	100.00
Between litter sizes	5	263.37	1.45	(negative)	(negative)
Between litters	20	181.74	1.07	(negative)	(negative)
Between breeds	13	354.91	18.15[3]	174.39	89.92
Within breeds	36	19.55		19.55	10.08

[3] $P < 0.001$.

TABLE 36. Number of young and mean birth weight of pure breeds borne by cross-bred does.

Litter size	Large race			Small race		
	Number of young	Mean birth weight, gms.	Estimated mean birth weight, gms.	Number of young	Mean birth weight, gms.	Estimated mean birth weight, gms.
1	2	76.0	84.3	1	61.0	59.4
2	6	73.3	75.7	8	56.9	55.0
3	19	74.4	71.1	14	50.1	52.5
4	3	77.7	68.0	5	56.2	50.9
5	3	55.0	65.7	2	46.5	49.6
6	7	62.1	63.8	5	49.6	48.6
Total and goodness of fit.	40		0.5201[3]	35		0.4413[2]

[2] $P < 0.01$; [3] $P < 0.001$.

(between litters), whereas 77.28 per cent of the variance is within litters, i.e. between and within breeds.

Table 36 shows the mean birth weights for each of the breeds within each litter size. The difference between the breeds is most clearly seen in the small litter sizes, but in no case does the weight of Polish reach a similar size to those of Large race. This applies not only to the means, but also to each litter in which young of the two breeds have been born simultaneously. The smallest difference is in two litters in which the mean weights of Polish are 46 and 46.5 gms. respectively, the mean weight of Large race being 55 gms. in both. A comparison of the observed weights with the corresponding weights in the normal pure breeds gives an indication of the modifications due to the changed uterine conditions (cf. Tables 24 and 27). The birth weight of Polish has increased considerably and is ten to fifteen gms. higher than the normal, whereas the weight of Large race has decreased and is in litter size 1 only 76 gms. against the normal weight of 109 gms. With an increasing number of young in the litter the weight approaches the normal, and in a couple of cases it is higher. The explanation of this is that the weights given in the table represent the means for the various numerical litter sizes, irrespective of their racial composition, and they can therefore not be directly compared with normal litters of the same number.

As for the previous groups the three different functions have been examined, but for each of the breeds separately. For Polish the function $y = 59.36 \cdot x^{-0.1113}$ is found to be the one which fits the observed data best, ($R = 0.4413$, $P < 0.01$), but there is no great difference between this and the exponential function

$y = 57.84 \cdot 1.030^{-x}$; ($R = 0.3947$, $P < 0.02$). The standard error is for the former $\binom{x}{2}$ 1.0979 and for the latter $\binom{x}{3}$ 1.1004. The straight line $y = 57.91 - 1.58\,x$ only just fits significantly with the observed values ($R = 0.3884$, $P < 0.05$). The agreement of the functions with the actual data is on the whole better for Large race. Thus $y = 84.31 \cdot x^{-0.1553}$ fits satisfactorily; ($R = 0.5201$, $P < 0.001$), and $y = 85.22 \cdot 1.056^{-x}$ has $R = 0.6004$ ($P < 0.001$). The standard errors are respectively $\binom{x}{2}$ 1.1195 and $\binom{x}{3}$ 1.1115. The straight line $y = 83.54 - 3.60\,x$ fits the observed values satisfactorily, ($R = 0.5868$, $P < 0.001$), within the interval of litter sizes one to six.

F. *Crossbreds borne by crossbreds.*

In order to make a comparison between the E-group and a pure F_1-generation, i.e. a generation from which the maternal influence has been eliminated, a few preliminary transplantations of fertilized "F_1-ova" to F_1-mothers have been made. Ten litters have been born, comprising a total of 21 young in litter sizes of one to four. The mean weight of the receptors was $3.04 \pm \pm 0.172$ kgms.

Because of the heterogeneity of the material it is difficult to draw any reliable conclusions from the comparison with the pure breeds borne by crossbreds. However, from Fig. 13, which shows the mean birth weight of the F_1 together with the weight of the two pure breeds borne by crossbreds, it is seen that the weight of litter sizes two, three, and four ranges between those of the pure breeds, although considerably nearer to that of Large race than to that of Polish. The mean weights of the different litter sizes is:

Litter size	1	(3 young)	79.3 gms.
„	2	(8 „)	65.9 „
„	3	(6 „)	67.2 „
„	4	(4 „)	72.0 „

An analysis of variance (Table 37) does not show any statistically significant difference between either litter sizes or litters. About 60 per cent of the computed variance of 118.47 is within litters, and 30 per cent is accounted for by differences between litters. If the material had been genetically homogenous, the whole of the variation would presumably be attributable to environmental differences (apart from any variation due to mutation), but it is in fact most likely that there is a genetically caused variation in addition to a variation due to the interaction of genes and environmental factors.

The agreement between the observed birth weights and those calculated from the power function has been tested, but owing to the limited material and the great variation between and within litters, it is not very good. The function is

VOL. 31 1950

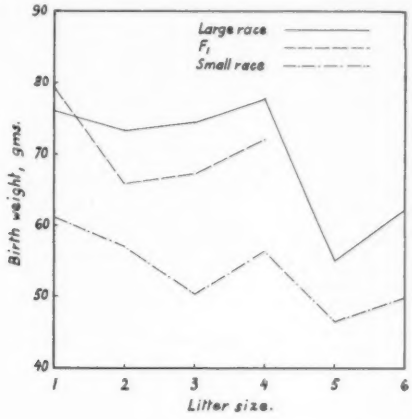


Fig. 13. Birth weight for F_1-young borne by F_1-mothers.

$y = 71.95 \cdot x^{-0.0565}$; ($R = 0.1659$, $P < 0.5$) with a standard deviation of $(\frac{x}{n})$ 1.1595. Examination of the relative birth weight (Table 37) shows different proportions of the variance attributable to the various factors. Thus the variance within litters is reduced to about one fifth, while the variance between litter sizes amounts to about two fifths of the calculated variance of 0.3463. The difference between the litters is barely significant ($P < 0.05$) whereas the difference between litter sizes falls short of significance, the variance ratio being 3.15.

COMPARISON OF THE RESULTS FROM THE DIFFERENT GROUPS.

In order to study the influence of the intra-uterine environment on the size of the fetus in rabbits numerous transplantations of fertilized ova of pure breeds as well as of "crossbreds" have been carried out. By subsequently com-

TABLE 37. Analysis of variance of birth weight of F_1-fetuses borne by F_1-does.

Source of variation	D. F.	Observed birth weight			Relative birth weight		
		Mean square	Variance ratio	Components of variance, %	Mean square	Variance ratio	Components of variance, %
Total	20	103.53			0.3089		
Between litter sizes .	3	150.77	1.05	11.90	1.0942	3.15	41.06
Between litters . .	6	143.37	2.08	29.92	0.3478	4.73[1]	37.71
Within litters . . .	11	68.92		58.18	0.0735		21.23

[1] $P < 0.05$.

paring the various experimental groups with normally born young of the same genetic constitution, it is possible to determine how much of the variation is attributable solely to differences in the uterine environments. In addition, however, by keeping the uterine environmental factor constant, these transplantation experiments provide a means of determining the importance of other sources of variation.

The variation in a population or sample is generally divided into three different components. The first is the variation due to genetic factors, the second is caused by environmental differences, and the third is attributed to the interaction of the "heritable" and environmental factors (WRIGHT 1935). MATHER (1949) divides the observed variation into a non-heritable portion (caused exclusively by environmental factors), a heritable portion, which owes its existence to the substitution of one allelomorph for another, and finally a third component, which is due to dominance and "arises from the differences between the expression of heterozygotes and the average of the two corresponding homozygotes". A more detailed analysis has been made by LUSH (1943, 1949), who besides retaining the division into the environmentally caused part and that which is due to interaction between environment and inheritance, subdivides the purely heritable variance into portions due to the additive effect of the genes, variations due to dominance, and variations due to epistasy. Such divisions of the variation are rather of a formal character and are connected with the definition of heritability. However, when it is desired to stress the importance of the part played by various non-genetic factors, a division based upon somewhat different principles is of course permissible. In the present investigations it is naturally of very great interest to elucidate how large a part of the variation is of a genetic nature, but to attempt to ascertain the origin of the various causes of genetic variations would lead too far from the problem under consideration. It has been emphasized in the survey of earlier investigations that the general interpretation of the mechanism of size inheritance is based on the hypothesis of additive inheritance, or as CASTLE (1922) expressed it, "blending inheritance". Further, it is generally agreed that size is influenced by a very large number of genes, whose effect, additive or cumulative, does not manifest itself exclusively in size, but simultaneously there is in all probability a physiological influence, the extent of which is not known, but which can be divided into several factors operating separately (CASTLE 1934, CASTLE *et al.* 1936). However, it must be regarded as probable that special genes operate physiologically by increasing the growth processes in those individuals which attain a higher weight, just as it appears reasonable to suppose that small size may be the result of a genetically caused retardation of the growth. Together with the genetico-physiological influence it is also necessary to take into consideration the purely physiological influence of the mother animal on the size of the fetus, operating through undernutrition or faulty nutrition, and which

VOL.
31
1950

may result in a smaller birth weight for larger litters. When the mother is pregnant with a large number of fetuses it is improbable under normal conditions, that there should be as much nutrient available for each individual fetus as in the smaller litters. Consequently in large litters the individual fetus is exposed to conditional undernourishment. At the same time, however, the space conditions in the uterus have a modifying (restricting) influence on the developmental potentiality of the individual fetus.

The question is of importance for an understanding of the way in which the variation is to be partitioned. One part of the physiological effect on the weight of the fetus is due to the genetic structure of the individual fetus and will consequently in an analysis be found in the heritable part of the variation. The other physiological influence on the size of the fetus is of a non-genetic nature. It would therefore be included in the non-heritable variation. Furthermore, an interaction between inheritance and environment cannot be left entirely out of consideration, but it is very difficult to determine the importance of this interaction.

In the determination of the influence of different factors on the birth weight it is necessary to consider the contingency of paternal sex-linked inheritance. The possibility exists that certain size genes are carried on the Y-chromosome, and it should then be feasible to determine the influence of these by finding genes for morphological characters, such as colour, with which these size genes might be linked. Incidentally CASTLE (1941) held the view that brown colour is generally linked with genes which have an increasing effect on size. If size genes are linked with colour genes to the Y-chromosome, it would be possible to reveal this linkage through selection experiments aiming at increasing the difference between the mean weights of the two sexes. Furthermore it is possible that a difference in size between reciprocal crosses is partially due to paternal sex-linked genes. If that were the case, it might be presumed *a priori*, that the male offspring of Small race buck × Large race doe would have a lower weight than those of the reciprocal cross. The figures in Table 1 show that the reverse has proved to be the case, and there is consequently no evidence for this supposition. Therefore it is at least equally important to examine the possibility of X-linked inheritance. If the size difference between the two sexes is caused by genes on the X-chromosome, the size difference would be due rather to purely dominant or recessive genes, and this conflicts with the general concept of the whole mechanism of size inheritance. On the other hand, it is quite possible that the maternal influence, which has been ascertained both by others and in this material, is of a cytoplasmic nature, for the possibility cannot entirely be ignored that cytoplasmic inheritance is to be found in mammals as has been shown for lower organisms, (cf. GOWEN *et al.* 1946, SONNEBAR 1948).

The division of the variation into portions is closely related to the treatment

of a quantitative character inherited according to a "polygenic system" (MATHER 1949). With a discontinuous variation within a population the individual variates fall into natural classes, and it is not necessary to state other measurements for the variation than the purely numerical relations between the various classes. However, somewhat different treatment must be adopted for material which shows continuous variation as do quantitative characters. For such characters it is necessary to resort to biometrical symbols to characterize the variation and the differences between various populations or samples and in order to express the heritability of the character. In the present work, the purpose of which is to determine the extent of the influence of the intra-uterine environment on the weight of the young at birth, where it is of importance to separate the purely materno-physiological influence from any cytoplasmic inheritance, the total variance will be divided into three main components. The first includes the heritable part of the variation (H), the second is the cytoplasmic portion (C), and the third component comprises the whole of that attributable to the environmental factors (E). The third component is subdivided into three parts, the first of which includes the influence of litter size, the second the influence due to intra-uterine environment, and the third part is the variation caused by environmental influence due to chance (error).

The total variance then has the following composition:

Heredity in the broad sense ..... σ^2_H

Cytoplasmic influence ..... σ^2_C

Environmental influence:

Litter size ..... σ^2_{El}

Intra-uterine environment ..... σ^2_{Eu}

Environment due to chance (error) ..... σ^2_{Er}

The relative variance can then be expressed by the equation $h^2 + c^2 + e^2_l + e^2_u + e^2_r = 1$, where h^2 represents the whole of the genetic variation or heritability in the broad sense (LUSH 1943, 1949), c^2 the cytoplasmic variation as a fraction of the phenotypic variance, etc.

For the sake of simplicity an index designation will be used for the variance. As above, *H* represents the genetic, *C* the cytoplasmic and *E* the environmental parts of the variance. For the individual groups the variance will be designated with *L* for Large race, *S* for Small race, *SL* for crossbreds between Small race ♂ and Large race ♀ and *LS* for the reciprocal cross. The variance for the different groups has the following composition:

VOL. 31 1950

A$_{I}$. Normal Large race ..... $_{L}\sigma^{2}_{H} + {}_{L}\sigma^{2}_{C} + {}_{L}\sigma^{2}_{El} + {}_{L}\sigma^{2}_{Eu} + {}_{L}\sigma^{2}_{Er} = \sigma^{2}_{T}$

A$_{II}$. Large race borne by Polish $_{L}\sigma^{2}_{H} + {}_{L}\sigma^{2}_{C} + {}_{L}\sigma^{2}_{El} + {}_{S}\sigma^{2}_{Eu} + {}_{L}\sigma^{2}_{Er} = \sigma^{2}_{T}$

B$_{I}$. Normal Polish ..... $_{S}\sigma^{2}_{H} + {}_{S}\sigma^{2}_{C} + {}_{S}\sigma^{2}_{El} + {}_{S}\sigma^{2}_{Eu} + {}_{S}\sigma^{2}_{Er} = \sigma^{2}_{T}$

B$_{II}$. Polish borne by Large race $_{S}\sigma^{2}_{H} + {}_{S}\sigma^{2}_{C} + {}_{S}\sigma^{2}_{El} + {}_{L}\sigma^{2}_{Eu} + {}_{S}\sigma^{2}_{Er} = \sigma^{2}_{T}$

C$_{I}$. Normal SL-crossbreds .. $_{SL}\sigma^{2}_{H} + {}_{L}\sigma^{2}_{C} + {}_{SL}\sigma^{2}_{El} + {}_{L}\sigma^{2}_{Eu} + {}_{SL}\sigma^{2}_{Er} = \sigma^{2}_{T}$

C$_{II}$. SL-crossbreds borne by Polish ..... $_{SL}\sigma^{2}_{H} + {}_{L}\sigma^{2}_{C} + {}_{SL}\sigma^{2}_{El} + {}_{S}\sigma^{2}_{Eu} + {}_{SL}\sigma^{2}_{Er} = \sigma^{2}_{T}$

D$_{I}$. Normal LS-crossbreds .. $_{LS}\sigma^{2}_{H} + {}_{S}\sigma^{2}_{C} + {}_{LS}\sigma^{2}_{El} + {}_{S}\sigma^{2}_{Eu} + {}_{LS}\sigma^{2}_{Er} = \sigma^{2}_{T}$

D$_{II}$. LS-crossbreds borne by Large race ..... $_{LS}\sigma^{2}_{H} + {}_{S}\sigma^{2}_{C} + {}_{LS}\sigma^{2}_{El} + {}_{L}\sigma^{2}_{Eu} + {}_{LS}\sigma^{2}_{Er} = \sigma^{2}_{T}$

E. Large race simultaneously with Polish } borne by crossbreds { $_{L}\sigma^{2}_{H} + {}_{L}\sigma^{2}_{C}$ / $_{S}\sigma^{2}_{H} + {}_{S}\sigma^{2}_{C}$ } $+ {}_{S+L}\sigma^{2}_{El} + {}_{(SL)}\sigma^{2}_{Eu} + {}_{S+L}\sigma^{2}_{Er} = \sigma^{2}_{T}$

F. LS-crossbreds SL-crossbreds } borne by crossbreds { $_{LS}\sigma^{2}_{H} + {}_{S}\sigma^{2}_{C}$ / $_{SL}\sigma^{2}_{H} + {}_{L}\sigma^{2}_{C}$ } $+ {}_{(LS)}\sigma^{2}_{El} + {}_{(LS)}\sigma^{2}_{Eu} + {}_{(LS)}\sigma^{2}_{Er} = \sigma^{2}_{T}$

In accordance with the examinations previously made of the influence of different factors on the birth weight, the calculations will be made with the litter size as the constant factor, i.e. within litter size, as a result of which a large part of the environmental portion of the variance, the σ^{2}_{El}-fraktion, will be left out of the calculations.

The part of the variance falling within litters is probably a criterion for measuring the average size of the heritable variation between young of the same litter. Only minor sources of error will be encountered, as the weighing of the young has taken place at the same time, and further the variation to be found within a litter seems, for the greater part, to be independent of the litter size, as has previously been mentioned. On the other hand, part of the variation to be found between litters will be caused by errors made during the experiments. There have been differences in the times of weighing the various litters, which in some cases may have resulted in either the whole litter or individual young of such a litter having taken nourishment before the weighing has taken place, with the result that the weight is somewhat higher than the actual birth weight. From autopsies within twenty-four hours after birth it has been found that the quantity of milk sucked by a young may be as much as 6—8 gms., or in the few cases examined, up to 10—12 per cent of the weight of the young.

Another part of the variation between litters is probably due to the fact that in some of the breeds the larger mothers show a tendency to bear rather heavier litters. However, with the distribution of the maternal weight found within the normal as well as within the transplanted groups this effect will be smoothed out. There may be other environmental factors having a different effect on the individual litters, but no doubt part of the variation is of a genetic nature.

Unfortunately the material does not allow the calculation of "repeatability" or the intraclass-correlation, as the mothers have only in very few cases had more than one litter.

In the following a detailed account will be given of the results which have been obtained by combining the different groups in various ways. By pooling the data from different groups it is possible to isolate the modification caused by changes in uterine environment. Further, by comparing groups developed under "uniform" uterine conditions it will in some cases be possible to separate the influence of genetic from cytoplasmic factors, if such exist.

VOL.
31
1950

I. DIFFERENCES IN UTERINE ENVIRONMENT.

$A_I + A_{II}$. Large race, normal and transplanted litters. A comparison of the variances for the normal and the transplanted groups is shown in Table 38. The part of the variance which in the normal group is due to differences between the races (cf. Table 23) has been added to the variance between litters (calculated on the sum of squares), as a result of which there is a slight alteration of the two "between" variances. From the table it appears that in the first place there is a considerably greater absolute variance in the experimental material than in the normal material, and secondly that the variance between litter sizes in the transplanted group is of very much the same magnitude as the total variance for normal young. Moreover, there is the peculiarity that the variation between and within litters in the experimental material amounts to only half, or less than half, of the variation in the normal material. In other words, the size of the fetuses is largely dependent upon the space available in the uterus. Further, these results show that a very large part of the variation to be found in a normal litter, i.e. the variation within litters, which is generally supposed to be caused principally by genetic factors, is greatly influenced by environmental factors. Consequently the statement made on page 67 that genetic factors are of major importance must be modified. In agreement with MACARTHUR (1949), who could not find any change in the coefficients of variation in a selection experiment on mice according as the mean weight increased, it has not been possible in these investigations to find any modification of the coefficients of variation when the average birth weight decreased. MACARTHUR writes: "This suggests that when genetic variation is reduced, plastic modifications increase to a compensating degree", and holds

TABLE 38. Comparison between the variances of normal and transplanted Large race fetuses.

Variance	Normal		Transplanted	
	actual	per cent	actual	per cent
Total	185.06	100.00	251.42	100.00
Between litter sizes	20.82	11.25	173.82	69.13
Between litters	89.30	48.25	35.14	13.98
Within litters	74.94	40.50	42.46	16.89

that "natural phenocopies of genetic weight differences" develop. This indicates that the variation within litters is made up of various components, the relative size of which is very difficult to determine. Besides variations exclusively due to the environment and purely genetic variations, there is a variation caused by the interaction of the different factors. It is not only different environmental factors which may be mutually dependent, but there is also a state of interdependence (generally called dominance) between different allelomorphs. For a character that is likely to be inherited according to a polygenic system allowance must be made for an interaction of non-allelomorphs, which may be by far the most important. The next most important cause of variation is probably due to interaction between polygenes and non-heritable factors. The reduction of the variance within litters which occurs when the litters develop under comparatively restricted uterine space conditions causes the influence of the purely environmental factors to decrease considerably, and the greater part of the variation within litters is attributable to genetic differences between litter mates and to the interaction of environment and inheritance, or expressed in an other way, to imitations of the gene effect caused by the environment.

The total variance for the normal young has the following composition: ${}_{L}\sigma^{2}_{H} + {}_{L}\sigma^{2}_{C} + {}_{L}\sigma^{2}_{El} + {}_{L}\sigma^{2}_{Eu} + {}_{L}\sigma^{2}_{Er} = \sigma^{2}_{T}$, whereas the variance for the transplanted young comprises the following components: ${}_{L}\sigma^{2}_{H} + {}_{L}\sigma^{2}_{C} + {}_{L}\sigma^{2}_{El} + {}_{S}\sigma^{2}_{Eu} + {}_{L}\sigma^{2}_{Er} = \sigma^{2}_{T}$. By pooling the data it is possible to calculate the extent of the modifying influence of the changed uterine environment on the development of Large race fetuses. In the analysis of variance (Table 39) the difference between the transplanted and the normal young will consequently be $\sigma^{2}_{LEu} - {}_{S}Eu$, which amounts to 78.70 or 33.70 per cent of a total variance of 233.52, calculated according to WINSOR and CLARKE's (1940) method. There is a somewhat abnormal distribution of the variates, which appears from the "negative variance" between litter sizes, but the difference between normal and transplanted litters is highly significant ($P < 0.001$), and moreover, the analysis of variance shows a highly significant difference between the litters within the two groups. The part of the variance for "between litters" is estimated at

TABLE 39. A1 + A11. Influence of changed uterine environment of the birth weight of Large race fetuses.

Source of variation	Observed birth weight				
	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	624	191.86		233.52	100.00
Between litter sizes	12	1470.34		(negative)	(negative)
Between normal-transplanted	5	3375.22	5.25[3]	78.70	33.70
Between litters	73	642.51	8.96[3]	83.11	35.59
Within litters	534	71.71		71.71	30.71

Source of variation	Relative birth weight				
	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	350	1.4258		1.5175	100.00
Between litter sizes	11	33.5680	1.79	0.5071	33.42
Between normal-transplanted	4	18.7305	20.07[3]	0.8134	53.60
Between litters	44	0.8935	16.73[3]	0.1436	9.46
Within litters	291	0.0534		0.0534	3.52

[3] $P < 0.001$.

35.59 per cent, and the variance for "within litters" is found to be 30.71 per cent of the total variance.

The immediate modifying influence of the alien uterine environment is shown in Table 40. The birth weight of single born young is reduced to 71.4 per cent of the normal weight, while it is further reduced for larger numbers of young in the litter (except in the case of litter size four, represented by only one litter, for which the normal weight is a little lower than would be expected, Table 24) and will probably go down to about 60 per cent in very large litters. A fetus weight of about 80 gms. seems (with one exception) to be the maximum size which Polish does can produce, but in the majority of cases the fetus is too large to survive the birth. Here again the largest fetus comprises the exception. Fig. 14 shows the calculated power functions for the two groups. In the normal material the average birth weight in the large litter sizes is about 55 gms. If it had been possible to obtain a corresponding litter size by transplantation of fertilized ova, the weight per young would probably have been about 30—35 gms. It has not been possible in these investigations to determine whether this is below the minimum size of viable young of Large race.

VOL. 31 1950

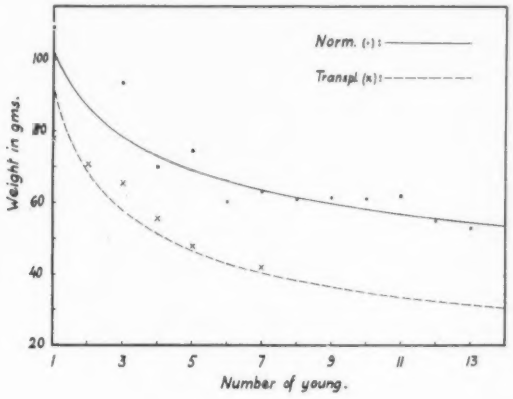


Fig. 14. Observed and estimated birth weight (y) of Large race in relation to litter size (x).

Normal: $y = 102.24\,x^{-0.2464}$.
Transplanted: $y = 91.42\,x^{-0.4197}$.

In the discussion of the influence of various factors on the birth weight it was pointed out that there is a very close relationship between the weight of the mother and the weight of the litter. The partial correlations between maternal weight and litter weight with litter size and gestation period constant were found to be 0.4448 for normal and 0.5338 for transplanted litters. Likewise it was ascertained that in the case of normal young there was a significant total

TABLE 40. Modification of birth weight due to difference in uterine environment. Transplanted as a percentage of normal.

Litter size	A. Large race		B. Small race		C. SL-cross-breds		D. LS-cross-breds		E. Pure breeds borne by crossbreds			
									Large race		Small race	
	Observed	Estimated	Observed	Estimated	Observed	Estimated	Observed	Estimated	Observed	Estimated	Observed	Estimated
1	71.4	89.4	106.5	121.4	—	87.4	(191.0)	159.2	69.7	82.5	116.0	117.6
2	—	79.2	132.7	130.5	81.0	85.2	130.8	146.2	—	87.7	130.7	121.3
3	70 0	73.8	138.5	136.1	97.3	83.9	138.2	139.0	79.7	91.0	111.3	123.5
4	79.3	70.2	150.3	140.3	—	83.1	130.9	134.1	111.0	93.4	135.8	125.1
5	64 3	67.6	135.5	143.6	—	82.4	135.2	130.5	73.8	95.4	117.1	126.4
6	63.0	65.5	145.3	146.4	72 2	81.8	114.9	127.6	103.3	97.0	127.8	127.4
7	—	63.7	—	148.7	71.0	81.4	124.5	125.1	—	98.3	—	128.3
8	—	62.3	147.2	150.8	—	81.0	—	123.1	—	99.5	—	129.0
9	—	61.0	—	152.6	—	80.6	—	121.3	—	100.6	—	129.7
10	—	59.9	—	154.3	—	80.3	—	119.7	—	101.6	—	130.4
11	—	58.9	—	155.9	—	80.0	—	118.3	—	102.5	—	130.9
12	—	58.1	—	157.3	—	79.8	—	117.1	—	103.3	—	131.4
13	—	57.3	—	158 6	—	79.5	—	115 9	—	104.0	—	131.9

correlation between the weight of the mother and the weight of the individual young. The corresponding correlation for the transplanted young was of the same magnitude, but not statistically significant because of the small number of variates in the group. In order to eliminate, at least partially, this influence of the maternal weight an analysis has been made, in which the relative weight of the young (the weight of the young as a percentage of the weight of the mother) is the variate. The result is shown in Table 39, from which it will be seen that, as in the previous analysis, there are significant differences between the transplanted and the normal individuals ($P < 0.001$). These differences entirely obscure the difference between litter sizes, which cannot, however, be left out of consideration, for 33.42 per cent of the calculated variance belongs to this source of variation. The variance both between and within litters is less in this analysis and in all does not amount to more than 13 per cent of the total variance.

By comparing the relative weight with the size of the litter a highly significant difference ($P < 0.001$) is found between the regression lines for the two groups. This is due to the fact that the regression for the transplanted young is negative and there is a highly significant correlation between the observed and the calculated values ($R = 0.7794$, $P < 0.001$), whereas the regression for the normal young must be assumed to be parallel to the x-axis ($R = 0.0403$, $P < 0.6$). The large difference between the intercepts of the lines is caused by the difference in the maternal weights of the two groups and this shows that the reduction of the birth weight is not directly proportional to the size of the mother. The regression lines are shown in Fig. 15. A linear regression has been used, a closer study of the coefficients of correlation having revealed that such a line is in this case more in agreement with the observations within the range of observed litter sizes than any of the previously discussed functions.

$B_I + B_{II}$. *Polish, normal and transplanted litters.* Table 41 shows the composition of the variance for the normal and the transplanted group of Polish young. In contrast to Large race, where the greatest variance was between litter sizes of transplanted young, there is a "negative variance" in this group. This causes the very large absolute and relative variances between litters. The variance found here is a little greater than the total variance for normal, and further, it is two and half times greater than the absolute variance between litters for the normal material. Within litters, the absolute variance is smallest for normal, while for the experimental group the relative variance is the smaller. Further it must be mentioned that the total variance for Polish amounts to only one third of the total variance for normal Large race, and the total variance for transplanted is a little less than one half of the variance for the correponding group of Large race. The absolute variance within litters is the same in each of the two transplanted groups.

VOL. 31 1950

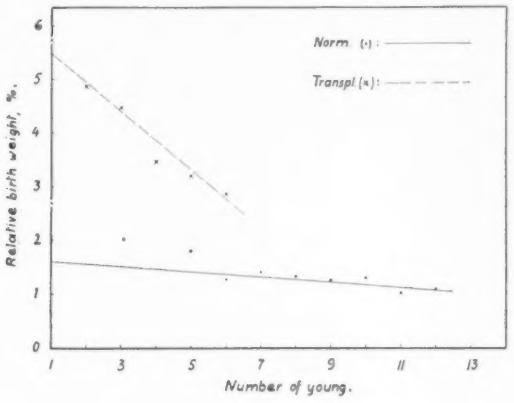


Fig. 15. Observed and estimated relative birth weight (v) of Large race in relation to litter size (x).

Normal: $v = 1.598 - 0.035\,x$.
Transplanted: $v = 6.028 - 0.549\,x$.

A treatment of both groups pooled gives a means of calculating the variance for differences in uterine environments for Polish fetuses, as was done for Large race, but in this case calculated for young whose normal size is about 50—60 per cent of the size of the Large race young. For the normal group the composition of the variance is ${}_S\sigma^2_H + {}_S\sigma^2_C + {}_S\sigma^2_{El} + {}_S\sigma^2_{Eu} + {}_S\sigma^2_{Er} = \sigma^2_T$, and for the experimental group ${}_S\sigma^2_H + {}_S\sigma^2_C + {}_S\sigma^2_{El} + {}_L\sigma^2_{Eu} + {}_S\sigma^2_{Er} = \sigma^2_T$, whence the difference between normal and transplanted can be expressed by $\sigma^2_{LEu - SEu}$. The results of the analysis of variance are shown in Table 42. The difference between transplanted and normal young is highly significant ($P < 0.001$), as is the difference between the litters within transplanted-normal ($P < 0.001$). The total variance amounts to 110.64, and 58.20 per cent of it is made up of the variance between and within litters, so that the remaining part, $\sigma^2_{LEu - SEu}$, which expresses the modification caused by differences in uterine environments, will be 46.25 or 41.80 per cent of the total variance.

The birth weights for the normal and the experimental material are shown in Table 27. The birth weight of the singly born young after transplantation is not significantly higher than that of the normal singly born young, but in

TABLE 41. Comparison between the variances of normal and transplanted Small race fetuses.

Variance	Normal		Transplanted	
	actual	per cent	actual	per cent
Total	66.99	100.00	117.09	100.00
Between litter sizes	6.34	9.46	(negative)	(negative)
Between litters	30.46	45.47	76.51	65.34
Within litters	30.19	45.07	40.58	34.66

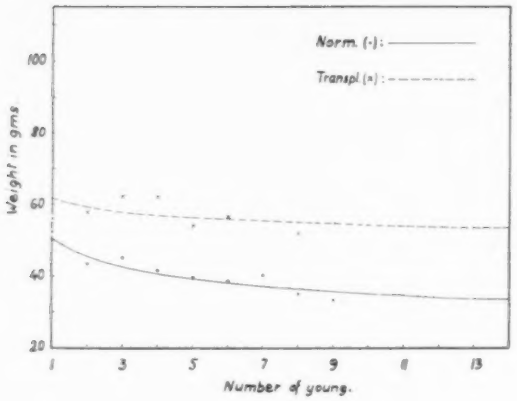


Fig. 16. Observed and estimated birth weight (y) of Small race in relation to litter size (x).

Normal: $y = 50.48\,x^{-0.1560}$.
Transplanted: $y = 61.27\,x^{-0.0516}$.

litter size two the difference between the two groups is clearly seen. The weight of the normal litters decreases slightly as the size of the litter increases, whereas the average weight of the transplanted litters first increases and then decreases slightly. On the average, the birth weight of Polish young increases to 140 per cent of the normal weight (Table 40), irrespective of the influence of the litter size. In other words, the increase in the birth weight of Polish

TABLE 42. $B_I + B_{II}$. Influence of changed uterine environment on the birth weight of Small race fetuses.

Source of variation	Observed birth weight				
	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	750	93.11		110.04	100.00
Between litter sizes	8	977.25		(negative)	(negative)
Between normal-transplanted	7	2341.16	13.75[a]	46.25	41.80
Between litters	164	170.28	5.49[a]	33.38	30.17
Within litters	571	31.01		31.01	28.03

Source of variation	Relative birth weight				
	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	376	0.4055		0.4898	100.00
Between litter sizes	7	2.9718		(negative)	(negative)
Between normal-transplanted	7	6.6247	7.82[a]	0.2299	46.94
Between litters	80	0.8472	13.64[a]	0.1978	40.38
Within litters	282	0.0621		0.0621	12.68

[a] $P < 0.001$.

VOL. 31 1950

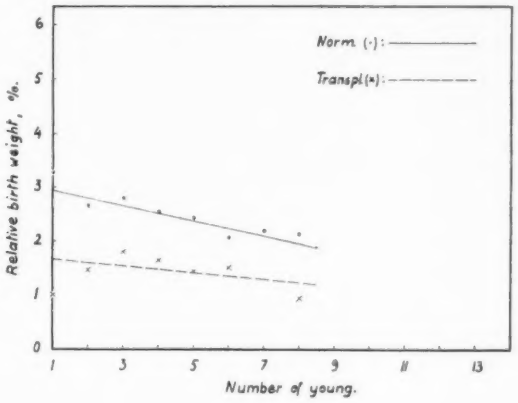


Fig. 17. Observed and estimated relative birth weight (v) of Small race in relation to litter size (x).

Normal: $v = 3.09 - 0.144\,x$.
Transplanted: $v = 1.74 - 0.063\,x$.

young developed in Large race does is of the same size as the decrease in the birth weight of Large race young developed in Polish does. Fig. 16 shows the power functions for the two groups.

In the discussion on the influence of various factors on birth weight it was shown that for transplanted litters there is a negative correlation between the weight of the mother and the weight of the litter, with litter size and gestation period constant. Likewise for this group there is a negative correlation between the weight of the mother and the weight of the young, whereas neither of these coefficients are significant for the normal material. For the other groups the corresponding coefficients were positive (cf. section D regarding one exception, Table 21). This difference in the correlations may be due to a purely physiological effect, whose origin is of a racial nature. However, if the birth weight is calculated in relation to the weight of the mother the size of the mother is partially eliminated, while at the same time it is possible to retain the variation within litters. The importance and extent of the different causes of variation in the relative birth weight are shown in the analysis of variance in Table 42. The difference between normal and transplanted young as well as the difference between litters remain highly significant ($P < 0.001$), whereas in these calculations also the difference between litter sizes is insignificant. The total variance is 0.4898, of which 46.94 per cent is represented by the variance for differences in uterine environments, and 40.38 per cent is between litters. In both cases the portions are somewhat larger than the corresponding ones in the other presented analysis of the different causes of variation, due to the considerably reduced variance within litters, which is only 12.68 per cent. A comparison with the results in Table 39 for Large race will show that the variance within litters is of the same absolute magnitude, but the difference between the transplanted and the normal young is more than three times less in Polish than in Large race, as is the total variance.

Fig. 17 shows the means of both groups for the relative birth weight in

relation to the litter size, and the regression lines within the interval where it is permissible to regard the relationship as linear. The difference between the lines is obvious and highly significant in so far as the intercept of the lines is concerned. On the other hand, it is doubtful if there is a real difference in the slopes of the lines, for it is barely significant ($P < 0.05$). The curve for transplanted has a distinct optimum for three or four young in the litter, but it is not possible on the basis of the comparatively few variates to conclude that the same should apply as shown by HALL and PIERCE (1934) for cats. It seems far more likely that the increase for litter size three in relation to the smaller sizes is accidental, and that no decrease in the relative birth weight takes place until the number of young in the litter exceeds eight.

$C_I + C_{II}$. *Crossbreds between Polish ♂ and Large race ♀, normal and transplanted litters.* The variances for normal SL-crossbreds and SL-crossbreds borne by Small race does have been compared in Table 43. It is seen that the absolute variance for the normal material amounts to only a little more than half the variance for the transplanted litters. In complete agreement with the results for Large race, the variance between litter sizes for transplanted is found to be of the same order as the total variance for the normal young (cf. p. 103), but at the same time the absolute variances are considerably smaller in the latter groups than in the former. This is a consequence of the difference in genetic structure between the two populations. However, the most important difference between the cross groups is in the variance within litters. This is probably due to the influence of the same factors as were discussed in connection with Large race. Besides the genetically caused variation between litter mates a considerable influence of the uterine environment (plus interaction) is observed in the normal group, whereas the greater part of the environmental influence is eliminated or restricted in its effect on fetuses which are developed in a comparatively restricted uterine space.

By the transplantation of the fertilized ova to Polish does only the intrauterine environment changes, the composition of the variance for normal crossbreds being ${}_{SL}\sigma^2_H + {}_{L}\sigma^2_C + {}_{SL}\sigma^2_{El} + {}_{L}\sigma^2_{Eu} + {}_{SL}\sigma^2_{Er} = \sigma^2_T$ and for the transplanted litters, ${}_{SL}\sigma^2_H + {}_{L}\sigma^2_C + {}_{SL}\sigma^2_{El} + {}_{S}\sigma^2_{Eu} + {}_{SL}\sigma^2_{Er} = \sigma^2_T$ whence the difference between the two groups is represented by $\sigma^2_{LEu - SEu}$. The results of the analysis of variance have been shown in Table 44. In this partition the difference between litter sizes is not significant. However, if the litter size forms a subclass of the partition, the difference is highly significant. Likewise, because of the distribution of the litter sizes in the two groups, there are only four degrees of freedom within litter sizes. Therefore the difference between normal and transplanted is not so obvious as might be expected if a larger number of litters had been studied. However, the difference is significant, and a division of the variance shows that 24.86 per cent of the total of 117.74 is

TABLE 43. Comparison between the variances of normal and transplanted SL-crossbred fetuses.

Variance	Normal		Transplanted	
	actual	per cent	actual	per cent
Total	89.39	100.00	155.86	100.00
Between litter sizes	30.74	34.39	80.83	51.86
Between litters	3.05	3.41	62.06	39.82
Within litters	55.60	60.20	12.97	8.32

exclusively caused by the difference in uterine environment. The variance between litters is of a similar size, while the smallest part of the variance is between litter sizes.

From Table 40 it is seen that the weight of the transplanted young is 70—80 per cent of the average birth weight of the normally born young. This agrees, not only with the analysis of variance, but also with the *a priori* assumption that the decrease for crossbred young caused by a restricted uterine space ought to be half the size of the decrease in the birth weight of Large race young

TABLE 44. $C_I + C_{II}$. Influence of changed uterine environment on the birth weight of SL-crossbred fetuses.

Source of variation	Observed birth weight				
	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	182	114.53		117.74	100.00
Between litter sizes	11	799.95	1.60	19.17	16.28
Between normal-transplanted .	4	499.33	3.03[1]	29.27	24.86
Between litters	25	164.58	3.94[2]	27.51	23.37
Within litters	142	41.79		41.79	35.49

Source of variation	Relative birth weight				
	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	155	1.6519		1.7596	100.00
Between litter sizes	11	18.4563	1.96	0.6936	39.42
Between normal-transplanted .	2	9.4396	7.72[2]	0.7374	41.91
Between litters	23	1.2229	24.22[2]	0.2781	15.80
Within litters	119	0.0505		0.0505	2.87

[1] $P < 0.05$; [2] $P < 0.001$.

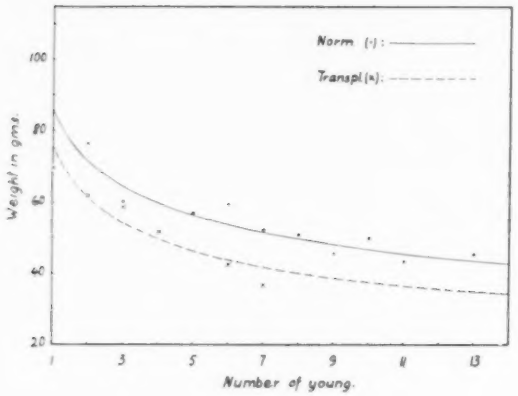


Fig. 18. Observed and estimated birth weight (y) of SL-crossbreds in relation to litter size (x).

Normal: $y = 86.02\,x^{-0.2625}$.
Transplanted: $y = 75.19\,x^{-0.2996}$.

developed in a similar uterine environment. The course of the power functions is shown in Fig. 18.

Information about the weights of the mothers of all the normal litters for use in the calculation of the relative weight is not obtainable. Details are availabie only for the mothers of 83 normal young. In the usual way, the analysis of variance (Table 44) has been carried out with the litter size as the first subclass, for which reason there does not appear in this case either to be any statistically significant effect of this factor. The difference between transplanted and normal is highly significant ($P < 0.001$), as is the difference between the litters ($P < 0.001$). The calculated parts of the variance are shown in the last column, and it appears from this that the variance between normal and transplanted contributes to the total variance just as large a share, as does the litter size. The variance within litters represents a negligible part of the total variation. Fig. 19 shows the regression lines for the relative birth weight in proportion to the litter size for the two groups. There is an obvious and statistically significant decrease in the relative birth weight for transplanted, but for normal it is more doubtful if there is any significant slope of the regression line. The regression coefficient of -0.024 is only 1.8 times the standard error, while the coefficient -0.259 is 5.3 times the standard error. A covariance analysis has shown that there is a highly significant difference between the slopes of the lines ($P < 0.001$), and further that the two lines differ significantly in their intercepts, i.e. the distance from the 0-point on the y-axis.

$D_I + D_{II}$. *Crossbreds between Large race ♂ and Polish ♀, normal and transplanted litters.* An examination of the different parts of the variance in the two groups (Table 45) shows how conspicuous is the difference between normal and transplanted with regard to the part that is accounted for by variations due to litter sizes, both absolutely and relatively. As far as the

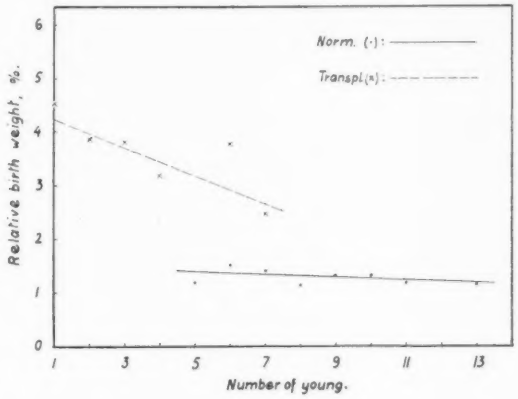


Fig. 19. Observed and estimated relative birth weight (v) of SL-crossbreds in relation to litter size (x).

Normal: $v = 1.51 - 0.024\,x$.
Transplanted: $v = 4.49 - 0.259\,x$.

normal young are concerned, there is a very strong resemblance to normal Polish, where the relative variance for the same class amounted to 9.46 per cent, but this does not apply in the case of transplanted. For Polish young borne by Large race does it was not possible to ascertain any modifying influence of the litter size (negative variance), but in this case the variance approaches 50 per cent of the computed total variance, in spite of the fact that in these two groups the size of the mothers has been the same. This means that cross fetuses are of such a large size in proportion to the developmental potentialities in the uterus that their growth is to some extent retarded, and even if their size is considerably above that of the normal crossbreds, it is by no means certain that the maximum size of crossbreds between Large race and Polish has been reached in these experiments. The only exception, if any, would be the young of litter size one. On the other hand it can be said that the weight of normal young in the largest observed litter sizes represents the minimum size of the young. The difference between singly born transplanted and the average weight in the largest litter size will thus be a criterion for the maximum modification.

By treating the two groups, normal and transplanted, pooled an estimate can

TABLE 45. Comparison between the variances of normal and transplanted LS-crossbred fetuses.

Variance	Normal		Transplanted	
	actual	per cent	actual	per cent
Total	50.66	100.00	109.75	100.00
Between litter sizes	3.72	7.34	48.51	44.20
Between litters	19.23	37.96	26.63	24.26
Within litters	27.71	54.70	34.61	31.54

be made of the influence exerted by a change of uterine environment on the birth weight of crossbreds between Large and Small race. In the case of the normal group the composition of the variance is, $_{LS}\sigma^2_H + {}_S\sigma^2_C + {}_{LS}\sigma^2_{El} + {}_S\sigma^2_{Eu} + {}_{LS}\sigma^2_{Er} = \sigma^2_T$ and in the transplanted group, $_{LS}\sigma^2_H + {}_S\sigma^2_C + {}_{LS}\sigma^2_{El} + {}_L\sigma^2_{Eu} + {}_{LS}\sigma^2_{Er} = \sigma^2_T$, so that the difference between the two groups is $\sigma^2_{LEu-SEu}$, i.e. the difference would be exclusively due to a non-cytoplasmic, maternal influence on the development of the fetuses. The analysis of variance is shown in Table 46. On the average the litter size exerts no influence (negative variance), but $\sigma^2_{LEu-SEu}$ represents about 70 per cent of the total variance. Less than one fifth of the variance is within litters.

Table 40 shows the mean birth weights for the two groups for transplanted as a percentage of normal. It will be seen that the birth weight of singly born young developed in Large race does is upwards of 40 per cent above the weight of normal (if the singly born young that weighed 32 gms. is left out of the calculations, if it is included the average increase will be 90 per cent over normal). Litter size six which has a rather high normal weight, deviates

TABLE 46. $D_I + D_{II}$. Influence of changed uterine environment on the birth weight of LS-crossbred fetuses.

Source of variation	Observed birth weight				
	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	210	129.71		167.48	100.00
Between litter sizes	6	807.51		(negative)	(negative)
Between normal-transplanted	7	1849.02	17.43[a]	115.64	69.05
Between litters	46	106.11	3.51[a]	21.57	12.88
Within litters	151	30.27		30.27	18.07

Source of variation	Relative birth weight				
	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	144	0.7742		1.1452	100.00
Between litter sizes	6	5.1349		(negative)	(negative)
Between normal-transplanted	5	12.1966	35.66[a]	0.9811	85.67
Between litters	28	0.3420	3.55[a]	0.0678	5.92
Within litters	105	0.0963		0.0963	8.41

[a] $P < 0.001$.

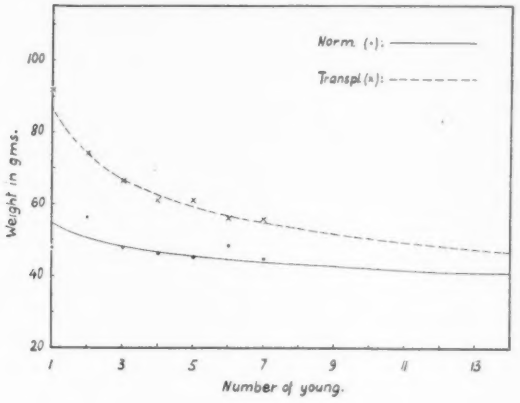


Fig. 20. Observed and estimated birth weight (y) of LS-crossbreds in relation to litter size (x).

Normal: $y = 54.21\, x^{-0.1088}$.
Transplanted: $y = 86.32\, x^{-0.2326}$.

a little. Here the weight of transplanted is only 15 per cent above normal. Generally, it can be said that the change of uterine environment has, for this type of cross, caused an increase in the weight of the newborn young of about 33 per cent above the normal weight. It must be pointed out, however, that the weight increase observed in this group is in direct contrast to the results for the other groups. In the previous groups the means were found to differ least in the case of the smallest litter sizes, while in this group the greatest difference is in the small litter sizes. This is attributed to the slight influence of the litter size on the weight of the young in the normal litters. However, as there is every indication that the normal material is not fully representative, the whole question will be taken up for discussion in a subsequent section. The power functions for the two groups are shown in Fig. 20.

The results of a comparison of the relative fetus weight in transplanted and normal LS-crossbreds are shown in Table 46. In this analysis "negative" variance for litter size is again found, but further, it is the difference between the two subclasses which is most conspicuous. The difference is highly significant ($P < 0.001$). Of the total variance, 85 per cent is accounted for by the difference in uterine environment, 6 per cent is attributable to differences between litters, and approximately 9 per cent is within litters. The correlation between the relative fetus weight and the litter size is shown in Fig. 21. There is a very marked and statistically significant difference between the regression lines for normal and transplanted with regard to both intercept and slope ($P < 0.001$). However, in direct contrast to the SL-crossbreds it is in this group doubtful whether the slope of the regression line for transplanted differs significantly from the horizontal. The coefficient of regression is $-0.059 \pm \pm 0.0275$, i.e. 2.15 times the standard error, while the coefficient for normal is -0.407 ± 0.0779 or 5.23 times the standard error and is consequently highly significant.

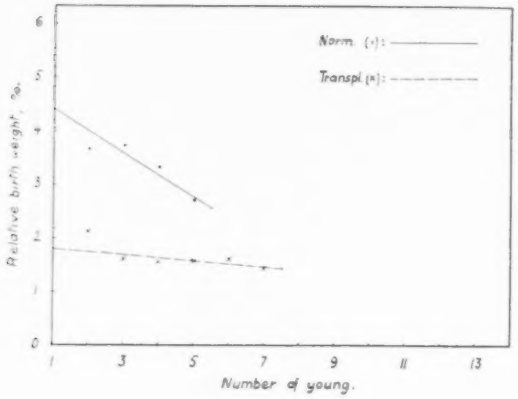


Fig. 21. Observed and estimated relative birth weight (v) of LS-crossbreds in relation to litter size (x).

Normal: $v = 4.81 - 0.407\,x$.
Transplanted: $v = 1.87 - 0.059\,x$.

II. CYTOPLASMIC DIFFERENCES.

For the pure breeds it is not possible to separate by ordinary methods the genetic from the cytoplasmic influence, but for the crosses between the breeds it is possible, by keeping the intra-uterine environment constant, to separate, at least to some extent, the two sources of variation. This presupposes, however, that the genetic constitution is the same in all the reciprocal crosses. It is generally assumed that the inheritance of size is governed by a polygenic system, and for which no special X- or Y-linked inheritance has been established. If this is true, then the variation may be treated so that two of the three components of the total variance are kept constant.

$C_I + D_{II}$. *Normal SL-crossbreds compared with transplanted LS-crossbreds.* The first comparison to be made is between normal crossbreds between Polish ♂ and Large race ♀ and reciprocal crossbreds likewise borne by Large race does. In the former case the composition of the variance is ${}_{SL}\sigma^2_H + {}_{L}\sigma^2_C + {}_{SL}\sigma^2_{El} + {}_{L}\sigma^2_{Eu} + {}_{SL}\sigma^2_{Er} = \sigma^2_T$, while in the latter group it is ${}_{LS}\sigma^2_H + {}_{S}\sigma^2_C + {}_{LS}\sigma^2_{El} + {}_{L}\sigma^2_{Eu} + {}_{LS}\sigma^2_{Er} = \sigma^2_T$, and on the supposition that there is no genetic difference between LS-crossbreds and SL-crossbreds the only difference in an analysis of variance of the two groups pooled will be σ^2_{LC-SC}. The result is shown in Tabel 47. The total variance is 103.60, and 42.18 or 40.71 per cent of it, accounted for by differences between litter sizes, is highly significant ($P < 0.001$). Within litter sizes the whole of the variation is caused by differences between litters, and the mean square for normal-transplanted, (which is theoretically composed of the variance within litters plus the variance between litters multiplied by $\frac{188}{38}$ plus the variance for normal-transplanted multiplied by $\frac{188}{17}$) is found to be smaller than the mean square within the same class. Consequently it is concluded that there is nothing

TABLE 47. $C_I + D_{II}$. Influence of difference in cytoplasm on birth weight of normal SL-crossbreds and transplanted LS-crossbreds.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	187	98.24		103.60	100.00
Between litter sizes	11	779.50	6.57[3]	42.18	40.71
Between normal-transplanted	5	53.53		(negative)	(negative)
Between litters	19	118.61	2.48[3]	13.55	13.08
Within litters	152	47.87		47.87	46.21

[3] $P < 0.001$.

to indicate that there is a cytoplasmic difference between the crossbreds which have their cytoplasm from either Large or Small race.

Further strong evidence that there is no cytoplasmic difference comes from the calculated functions, which for the normal SL-crossbreds is $y = 86.02 \cdot \cdot x^{-0.2625}$ and for transplanted LS-crossbreds is $y = 86.32 \cdot x^{-0.2326}$. There is no difference between these two. Similarly, there is no difference between the linear regressions of the relative birth weight on the size of the litter with regard to both the intercepts and the slopes (Table 48).

$C_{II} + D_I$. *Transplanted SL-crossbreds compared with normal LS-crossbreds.* A second means of comparing the groups, so that the genetic and the environmentally caused parts of the variance are kept constant, is by comparing normal crossbreds between Large race ♂ and Polish ♀ with the reciprocal crossbreds borne by Polish does. For the normal crossbreds, the composition of the variance is ${}_{LS}\sigma^2_H + {}_{S}\sigma^2_C + {}_{LS}\sigma^2_{El} + {}_{S}\sigma^2_{Eu} + {}_{LS}\sigma^2_{Er} = \sigma^2_T$ and for the transplanted ${}_{SL}\sigma^2_H + {}_{L}\sigma^2_C + {}_{SL}\sigma^2_{El} + {}_{S}\sigma^2_{Eu} + {}_{SL}\sigma^2_{Er} = \sigma^2_T$. Again assuming that there is no genetic difference and that the does which have borne the young are so representative of the Small race that there is no difference between the groups as far as the mothers are concerned, the difference between normal

TABLE 48. $C_I + D_{II}$. Analysis of difference in regression of relative birth weight on litter size for normal SL-crossbreds and transplanted LS-crossbreds.

Source of variation	D. F.	Remainder mean square	Variance ratio
Total	159	0.0814	
Difference between intercepts on Y-axis	1	0.2223	2.76
Remainder	158	0.0805	
Difference between slopes of regression lines	1	0.1586	1.98
Error	157	0.0800	

TABLE 49. $C_{II} + D_I$. Influence of difference in cytoplasm on birth weight of transplanted SL-crossbreds and normal LS-crossbreds

Source of variation	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	205	94.09		98.96	100.00
Between litter sizes	6	1097.61	2.41	21.82	22.05
Between normal-transplanted .	6	455.39	3.51[2]	20.56	20.78
Between litters	52	129.65	5.66[3]	33.68	34.03
Within litters	141	22.90		22.90	23.14

[2] $P < 0.01$; [3] $P < 0.001$.

and transplanted pooled is, as in the preceding comparison, $\sigma^2_{LC - SC}$. The analysis of variance is shown in Table 49, from which it is seen that in this case there is a significant difference between normal and transplanted ($P < 0.01$). Of the total calculated variance of 98.96, 20.78 per cent is between the two groups, i.e. the influence of cytoplasmic differences between Large race and Polish. This result, however, is in direct conflict with the result of the previous comparison in which no cytoplasmic effect was shown.

The fact that no difference could be ascertained between the functions for normal and transplanted was regarded in the above as a proof of the assumption of non-existence of cytoplasmic inheritance. A disagreement between the functions might therefore in this case be regarded as evidence of a cytoplasmic effect. In the case of the transplanted SL-crossbreds the function is $y = 75.19 \cdot \cdot x^{-0.2996}$, while in the normal LS-crossbreds it is $y = 54.21 \cdot x^{-0.1088}$. In the former there is agreement between the observed and the calculated values, $R = 0.7122$, and in the latter, $R = 0.2493$. The cause of the poor agreement in the case of the normal has previously been discussed, but further it must be emphasized that in all probability there have been certain extraneous, possibly even some intra-uterine, but nevertheless uncontrollable factors, which have contributed to the low birth weights in the smallest litter sizes of this group. The "normal" transplantations have shown that it is not difficult to obtain twice as high a birth weight in litter size one as has been observed, and further the transplantation experiments have proved that Polish does are quite capable of bearing larger young. The problem of cytoplasmic inheritance in rabbits can not be considered as definitely solved, however, by the experimental results which have been presented here. In order to draw final conclusions the material has to be extended.

An examination of the difference between the course of the straight regression lines for the relative birth weight shows a slightly significant difference

VOL. 31 1950

TABLE 50. $C_{II} + D_I$. Analysis of difference in regression of relative birth weight on litter size for transplanted SL-crossbreds and normal LS-crossbreds.

Source of variation	D. F.	Remainder mean square	Variance ratio
Total	138	0.3992	
Difference between intercepts on Y-axis	1	2.5920	6.76[1]
Remainder	137	0.3832	
Difference between slopes of regression lines	1	1.0600	2.80
Error	136	0.3782	

[1] $P < 0.05$.

between the intercepts of the lines ($P < 0.05$), whereas there is no difference in the slopes of the lines (Table 50).

F. Comparison between reciprocal crossbreds borne by crossbreds. Another possible means of distinguishing between cytoplasmic influences which may be exerted by the two breeds is through a consideration of the small experimental group comprising F_1 borne by F_1-mothers. If once again it is presumed that there are no genetical differences between the reciprocal crossbreds, the difference between them when developed in precisely the same uterine environment will be exclusively accounted for by cytoplasmic dissimilarities. In order to solve the problem satisfactorily, however, it is imperative that young within the same litter by Large race ♂ and Polish ♀, and *vice versa* can be distinguished. This may be done, for example, by breeding two strains of Large race which do not differ in size but only with regard to morphological characters. It has been attempted in these preliminary experiments to transplant ova from Vienna Blue ♂ × Polish ♀ simultaneously with ova from Polish ♂ × × Flemish Giant ♀, but unfortunately only the former cross has resultated in young being born.

The results obtained in this group will not be discussed in detail, but it may be mentioned that it has not been possible to detect any difference in the birth weight within litter sizes between the two types of crossbreds. The mean for LS-crossbreds (litter sizes two and three) is 66.1 gms., and the mean for SL-crossbreds (same litter sizes) is 66.7 gms. In both cases three litters comprising a total of seven young have been observed. These results also do not indicate any cytoplasmic differences.

III. GENETIC DIFFERENCES.

$A_I + B_{II}$. Comparison between normal Large race and transplanted Polish. In the case of Large race the composition of the variance was ${}_L\sigma^2_H + {}_L\sigma^2_C + {}_L\sigma^2_{El} + {}_L\sigma^2_{Eu} + {}_L\sigma^2_{Er} = \sigma^2_T$, and in transplanted Polish, ${}_S\sigma^2_H + {}_S\sigma^2_C +$

TABLE 51. $A_I + B_{II}$. Influence of difference in genetic structure on the birth weight of normal Large race and transplanted Small race.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	610	174.27		175.95	100.00
Between litter sizes	12	1197.40	1.11	2.53	1.44
Between normal-transplanted	6	1078.58	1.50	11.12	6.32
Between litters	66	721.10	10.02[a]	90.30	51.32
Within litters	526	72.00		72.00	40.96

[a] $P < 0.001$.

$+ {}_S\sigma^2_{El} + {}_L\sigma^2_{Eu} + {}_S\sigma^2_{Er} = \sigma^2_T$. Consequently the difference between the two groups within litter sizes when treated as a whole must represent the genetic (and cytoplasmic) difference. At the same time there is a certain interaction between genic and non-genic factors, whose positive influence (increasing the birth weight) is most marked in the case of Polish borne by Large race does for which combination the potentialities for development are most favourable. On the other hand, it is possible that this interaction has a decreasing effect on the Large race fetuses. The maximum difference between Large race and Polish in a constant uterine environment is the difference between the mean weights of normal Large race in litter size one and of Polish in the largest litter size observed, i.e. eight. The weight of the former is 109.0 gms. and of Polish, 51.9 gms., i.e. 47.60 per cent of that of Large race. The genetic difference is not very clear when the two groups are pooled. The analysis of variance (Table 51) shows that only 6.32 per cent of a total variance of 175.95 can be attributed to this cause of variation, whereas a little more than one half of the total variance is accounted for by differences between the litters and 41 per cent is due to differences within litters.

$A_{II} + B_I$. *Comparison between transplanted Large race and normal Polish.* As in the preceding comparison, an examination of the variation of the birth weight within transplanted Large race in relation to the variation within normal Polish will take the form of an estimation of the genetically caused difference between the two pure breeds. In the case of transplanted Large race the composition of the variance is ${}_L\sigma^2_H + {}_L\sigma^2_C + {}_L\sigma^2_{El} + {}_S\sigma^2_{Eu} + {}_L\sigma^2_{Er} = \sigma^2_T$ and in normal Polish, ${}_S\sigma^2_H + {}_S\sigma^2_C + {}_S\sigma^2_{El} + {}_S\sigma^2_{Eu} + {}_S\sigma^2_{Er} = \sigma^2_T$, so that the difference is to be found in the genetic (and cytoplasmic) part of the variation. In this case, however, the part of the variance due to environmental differences is only to be regarded as constant when it is calculated within litter sizes. The size of the litter has a very considerable modifying effect on the birth weight in Large race borne by Polish, while in normal Polish (as in normal Large

VOL. 31 1950

TABLE 52. $A_{II} + B_I$. Influence of difference in genetic structure on the birth weight of transplanted Large race and normal Small race.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	764	100.51		105.86	100.00
Between litter sizes	8	2146.97		(negative)	(negative)
Between normal-transplanted	6	2374.78	14.91[3]	43.44	41.03
Between litters	171	159.27	5.09[3]	31.11	29.39
Within litters	579	31.31		31.31	29.58

[3] $P < 0.001$.

race) it is of minor importance. The result of the comparison is shown in Table 52. As is to be expected, the total variance is considerably smaller than in the comparison between normal Large race and transplanted Polish, but what is most surprising is that in this case the variation caused by genetic dissimilarities amounts to 41.03 per cent of the whole of the calculated variance. Relatively, this is about seven times as large as in the last comparison (about four times as large absolutely). This increase means that a far smaller part of the variance within the two groups is to be found between and within litters. It therefore appears that the genetic difference between the two breeds manifests itself most clearly if the uterine space is inadequate to permit any considerable interaction between genetic and non-genetic influences.

$A_I + C_I$. *Comparison between normal Large race and normal SL-crossbreds.* The comparison between normal Large race young and normal SL-crossbreds will indicate the genetic variance between a P-generation and the F_1-generation. For the crossbreds, the composition of the variance is ${}_{SL}\sigma^2_H + {}_{L}\sigma^2_C + {}_{SL}\sigma^2_{El} + {}_{L}\sigma^2_{Eu} + {}_{SL}\sigma^2_{Er} = \sigma^2_T$, and consequently the genetic portion will be the only part which is not identical in the two groups. From Table 53, which

TABLE 53. $A_I + C_I$. Influence of difference in genetic structure on the birth weight of normal Large race and normal SL-crossbreds.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	656	185.81		188.28	100.00
Between litter sizes	12	1811.53	1.13	4.10	2.18
Between groups	9	1604.09	2.10[1]	28.12	14.94
Between litters	58	764.33	10.66[3]	84.34	44.79
Within litters	577	71.72		71.72	38.09

[1] $P < 0.05$; [3] $P < 0.001$.

shows the different causes of variation, it is seen that by far the greater part of the variation is due to differences between and within litters. The variance for the genetic difference between Large race and crossbreds amounts to 14.94 per cent of the total. The difference is significant ($P < 0.05$) in contrast to the difference calculated for the genetic difference between Large and Small race borne by Large race does.

$A_{II} + C_{II}$. *Comparison between transplanted Large race and transplanted SL-crossbreds.* A comparison between Large race and SL-crossbreds, both borne by Polish does, will, as in the preceding comparison, provide an estimate of the genetic difference, although in this instance the fetuses of both groups have developed under exceedingly restricted uterine conditions. The composition of the variance for the transplanted SL-crossbreds is, ${}_{SL}\sigma^2_H + {}_{L}\sigma^2_C + {}_{} + {}_{SL}\sigma^2_{El} + {}_{S}\sigma^2_{Eu} + {}_{SL}\sigma^2_{Er} = \sigma^2_T$. The birth weight has in fact been so modified in these groups that it is not possible to ascertain any differences whatever, as may be seen from Table 24 and Table 30. The partition of the variation between the different causes (Table 54) shows a significant difference between litter sizes ($P < 0.01$) and a highly significant difference between the litters ($P < 0.001$), but no difference between the groups. This means that the part of the variance, which according to this method of calculation, can be ascribed to genetic differences, does not amount to more than 1.31 per cent of the total variance. On the other hand, the variance for differences between litter sizes amounts in this case to 60.51 per cent, while for the two normal groups it was not more than 2.18 per cent. In the latter case particularly the differences between and within litters have had the greatest influence on the variations in birth weight.

$A_I + D_{II}$. *Comparison between normal Large race and transplanted LS-crossbreds.* The calculation of the difference between normal Large race and crossbreds between Large race ♂ and Polish ♀ gives a measure of the genetic difference between Large race and crossbreds which are reciprocal to those discussed in the two preceding sections. In the case of LS-crossbreds borne

TABLE 54. $A_{II} + C_{II}$. Influence of difference in genetic structure on the birth weight of transplanted Large race and transplanted SL-crossbreds.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	150	182.75		200.17	100.00
Between litter sizes	6	2813.02	14.04[2]	121.12	60.51
Between groups	5	200.29	1.20	2.63	1.31
Between litters	40	167.15	5.81[3]	47.66	23.81
Within litters	99	28.76		28.76	14.37

[2] $P < 0.01$; [3] $P < 0.001$.

VOL. 31 1950

TABLE 55. $A_I + D_{II}$. Influence of difference in genetic structure on the birth weight of normal Large race and transplanted LS-crossbreds.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	624	172.56		174.75	100.00
Between litter sizes	12	1549.46	1.80	14.36	8.22
Between normal-transplanted	6	858.84	1.29	5.86	3.35
Between litters	69	665.92	9.41[3]	83.80	47.95
Within litters	537	70.73		70.73	40.48

[3] $P < 0.001$.

by Large race does the composition of the variance is ${}_{LS}\sigma^2_H + {}_{S}\sigma^2_C + {}_{LS}\sigma^2_{El} + {}_{L}\sigma^2_{Eu} + {}_{LS}\sigma^2_{Er} = \sigma^2_T$. The result of the examination is shown in Table 55, from which it is seen that there are no significant differences between either litter sizes or groups. By far the greatest part of the variation is accounted for by differences between both the litters and the individuals within the same litter. The partitioning of the variance shows that 8.22 per cent of a total variance of 174.75 is due to differences between litter sizes and 3.35 per cent is accounted for by genetic differences between the young of the two groups. The remainder is due to causes which reside within the groups. A comparison with the analogous examination of Large race and Polish shows that the genetic part of the variance is 50 per cent smaller in this case than in the other two. On the whole this smaller variance is presumably due to the fact that the influence of the interaction is exerted through an increase in the size of the crossbred fetuses because of the larger uterus than is normal for crossbreds of this kind. On the other hand it is difficult to explain the difference between the result of this analysis and the result of $A_I + C_I$, where the genetic part amounted to approximately 15 per cent of a total variance of the same magnitude. This is the more remarkable as it has not been possible to ascertain any cytoplasmic difference between the groups C_I and D_{II}.

$A_{II} + D_I$. Comparison between transplanted Large race and normal LS-crossbreds. The following comparison between Large race borne by Polish does and normal LS-crossbreds (also borne by Polish does), corresponds to the preceding comparison in so far as it provides a means of estimating the genetic difference between Large race and crossbreds, although in this case the development of the fetuses has taken place under the reverse uterine conditions. There is a highly significant difference ($P < 0.001$) between the two groups, while the difference between the litters is also significant ($P < 0.001$), but both the absolute and the relative variance, not only between but also within litters, is smaller than the variance between the groups (Table 56). It should be

TABLE 56. $A_{II} + D_I$. Influence of difference in genetic structure on the birth weight of transplanted Large race and normal LS-crossbreds.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	210	125.50		133.70	100.00
Between litter sizes	6	1856.65	2.48	36.73	27.47
Between normal-transplanted	6	749.38	6.42[3]	38.98	29.16
Between litters	50	116.71	3.54[3]	24.99	18.69
Within litters	148	33.00		33.00	24.68

[3] $P < 0.001$.

mentioned in passing that a partition of the material with litter size as a subclass would likewise show significant differences between litter sizes. The division used, however, shows that 29.16 per cent of a total variance of 133.70 must be attributed to genetic dissimilarities between Large race and crossbreds, which is considerably more than found in the previous comparison. Here again it will be seen that the genetic difference manifests itself most clearly under uterine conditions which are so restricted that the interaction between genetic and non-genetic agencies is unable to express itself, as has already been discussed in the comparison of transplanted Large race and Polish.

$B_I + C_{II}$. *Comparison between normal Polish and transplanted SL-crossbreds.* For normal Polish the variance has the following composition: ${}_S\sigma^2_H + {}_S\sigma^2_C + {}_S\sigma^2_{El} + {}_S\sigma^2_{Eu} + {}_S\sigma^2_{Er} = \sigma^2_T$, while for transplanted SL-crossbreds it is as follows: ${}_{SL}\sigma^2_H + {}_L\sigma^2_C + {}_{SL}\sigma^2_{El} + {}_S\sigma^2_{Eu} + {}_{SL}\sigma^2_{Er} = \sigma^2_T$. The differences within litter sizes between the two groups therefore expresses the genetic difference between pure Polish and crossbreds between Polish ♂ and Large race ♀ when the cytoplasmic differences between the groups are left out of consideration. The result of the examination (Table 57) is that statistically

TABLE 57. $B_I + C_{II}$. Influence of difference in genetic structure on the birth weight of normal Small race and transplanted SL-crossbreds.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	759	90.98		93.43	100.00
Between litter sizes	8	1952.72	1.33	5.73	6.13
Between normal-transplanted	6	1469.00	9.03[3]	25.78	27.59
Between litters	173	162.67	5.65[3]	33.12	35.45
Within litters	572	28.80		28.80	30.83

[3] $P < 0.001$.

VOL. 31 1950

significant differences exist between the groups within litter sizes ($P < 0.001$), the calculated portion of the variance being 27.59 per cent of a total variance of 93.43. Within the groups, when the variance is partitioned between litters and within litters, each of the two portions is a little larger than between the groups, but it may be said that each of the three causes of variation contributes approximatively one third of the variance which is to be found within litter sizes. By comparing the figures of this analysis with the results of the preceding calculation it is seen that in the first place they confirm the observation that genetic dissimilarities manifest themselves most perceptibly in a comparatively restricted uterine environment, while further they show that the genetic portion of the variance in this analysis is in complete agreement with the portion calculated in the preceding analysis as relative variance.

$B_{II} + C_I$. *Comparison between transplanted Polish and normal SL-crossbreds.* As has been done in the calculations for Large race and Polish or crossbreds so may the method of calculating the genetic difference between pure Polish and crossbreds be carried out in the opposite way. The only difference between the two analyses is that in the one case the fetuses have developed in a spacious uterine environment and in the other case the development has taken place under restricted conditions. The result of this calculation is shown in Table 58 and further confirms the observation that the genetic differences have difficulty in asserting themselves under favourable developmental conditions. It has not been possible to show any differences within litter sizes between the two groups, from which it follows that a calculation of the variance for "between groups" would give "negative variance". The result is entirely in agreement with that of $A_I + D_{II}$, where it was shown that the birth weight of crossbreds might be increased under spacious uterine conditions. In the preceding case the birth weight increased to the weight found in normal Large race, while in this instance the birth weight of Polish is modified to such an extent that it does not differ from that of normal SL-crossbreds. This result can also be seen from Table 27 and Table 30, which show the average birth

TABLE 58. $B_{II} + C_I$. Influence of difference in genetic structure on the birth weight of transplanted Small race and normal SL-crossbreds.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	173	109.66		127.64	100.00
Between litter sizes	11	655.83	2.69[1]	40.27	31.55
Between normal-transplanted	5	139.14		(negative)	(negative)
Between litters	16	243.61	4.80[3]	36.57	28.65
Within litters	141	50.80		50.80	39.80

[1] $P < 0.05$; [3] $P < 0.001$.

weight of each litter size. There is a difference in the birth weight of twinborn young, which, however, is evidently not so great that it can affect the result of the analysis of variance, since for litter sizes three to eight the weight of transplanted Polish is in several cases higher.

$B_I + D_I$. *Comparison between normal Polish and normal LS-crossbreds.* The comparison between normal Polish on the one hand and normal crossbreds between Large race ♂ and Polish ♀ on the other is a comparison between young developed under uniform uterine conditions, and in which the size characters inherited through the mother, whether cytoplasmic or genic, are the same. The composition of the variance is for normal Polish, $_S\sigma^2_H + {}_S\sigma^2_C + {}_S\sigma^2_{El} + {}_S\sigma^2_{Eu} + {}_S\sigma^2_{Er} = \sigma^2_T$, while for normal LS-crossbreds it is, $_{LS}\sigma^2_H + {}_S\sigma^2_C + {}_{LS}\sigma^2_{El} + {}_S\sigma^2_{Eu} + {}_{LS}\sigma^2_{Er} = \sigma^2_T$. Genetic dissimilarities within the breed are ignored. Consequently the whole of the difference expresses the purely genetic difference between pure Polish and crossbreds which have received one half of their size genes from Large race through the sire. It has not been possible in this comparison (Table 59) to ascertain that the litter size has any statistically significant influence on the birth weight, while on the other hand both the difference between the two groups (i.e. the genetically caused difference) and the difference between litters are highly significant ($P < 0.001$). In this material the smallest absolute total variance, 71.18, is found, of which between 82 and 83 per cent is within the two groups, distributed equally between and within litters. The hereditary influence is calculated to be 7.87 or 11.05 per cent of the total, and is thus considerably smaller than the portion found in the analogous comparison between Large race and Polish ♂ × Large race ♀, both groups normal.

$B_{II} + D_{II}$. *Comparison between transplanted Polish and transplanted LS-crossbreds.* The next comparison to be made is between the two transplanted groups of Polish and LS-crossbreds. Here again the only genetic differences are those due exclusively to the sires. Furthermore, however, the development of

TABLE 59. $B_I + D_I$. Influence of difference in genetic structure on the birth weight of normal Small race and normal LS-crossbreds.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	819	70.09		71.18	100.00
Between litter sizes	8	966.60	1.75	4.54	6.38
Between groups	7	552.60	3.71[2]	7.87	11.05
Between litters	183	149.13	5.00[3]	28.96	40.69
Within litters	621	29.81		29.81	41.88

[2] $P < 0.01$; [3] $P < 0.001$.

VOL. 31 1950

TABLE 60. $B_{II} + D_{II}$. Influence of difference in genetic structure on the birth weight of transplanted Small race and transplanted LS-crossbreds.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	141	107.55		110.39	100.00
Between litter sizes	7	592.37	2.04	17.05	15.44
Between groups	6	289.74	1.42	8.43	7.64
Between litters	27	204.26	5.48[2]	47.64	43.16
Within litters	101	37.27		37.27	33.76

[2] $P < 0.01$.

both groups has taken place in an "unnatural" uterine environment, so that various interactions may have had an effect. Returning to the birth weights of the two groups, shown in Table 27 and Table 33, it is seen that for these groups also there is an important difference only in litter sizes one and two, whereas for larger litters there are no great differences. This is confirmed by the analysis of variance (Table 60), which shows no significant difference between either litter sizes or the two groups. The variation caused by genetic differences is here found to be 7.64 per cent of a total of 110.39 or somewhat less than the corresponding portion in the preceding comparison ($B_I + D_I$). With regard to absolute variance it is slightly larger, but so for that matter is the whole of the absolute variance. This is probably explained by the fact that in this case the uterine conditions have offered greater scope for the development of variation between and within the litters than have those of Polish. With this exception the two last analyses are in complete agreement with the earlier ones, which have shown a highly significant genetic difference between groups when the development of the young has taken place in Small race does, but no significant difference between the groups when this development has been in Large race does. The only exception which has occurred was for the comparison of Large race with SL-crossbreds, borne by either Large race (normal) or Polish (transplanted) mothers.

E. Comparison between Small and Large race, borne by crossbreds simultaneously. The last comparison to be made is between the two pure breeds, borne by crossbreds after simultaneous transplantation of fertilized eggs. The composition of the variance for this material is $\left.\begin{matrix} {}_L\sigma^2_H + {}_L\sigma^2_C \\ {}_S\sigma^2_H + {}_S\sigma^2_C \end{matrix}\right\} + S + H\sigma^2_{El} +$

$+ (SL)\, LS\sigma^2_{Eu} + S + H\sigma^2_{Er} = \sigma^2_T$.

The difference between the two breeds will therefore not be the purely genetic difference, but one which is caused by both genetic and possible cytoplasmic dissimilarities. The analyses of variance (Table 35) showed no difference be-

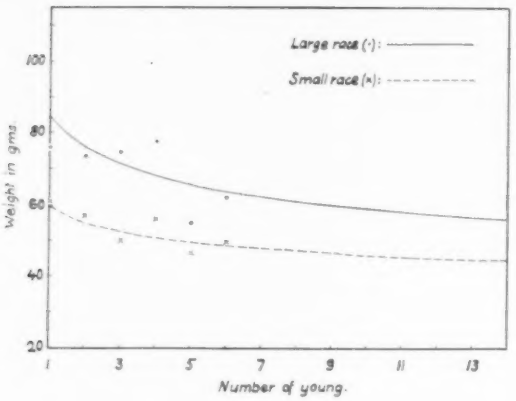


Fig. 22. Observed and estimated birth weight (y) in relation to litter size (x) for Large and Small race, borne by crossbreds simultaneously.

Large race: $y = 84.31\ x^{-0.1553}$.
Small race: $y = 59.36\ x^{-0.1113}$.

VOL. 31 1950

tween litter sizes or litters, but that 90 per cent of the variation was caused by racial differences. It is most probably that these differences are accounted for entirely by genetic factors. The power functions are shown in Fig. 22, while Table 40 shows the modification.

An examination of the relative weight does not reveal any difference between either litter sizes or litters (Table 61). The difference between the breeds appears equally clearly as from the absolute birth weight ($P < 0.001$). The division of the variance once again shows "negative variance" for litter sizes, while 7.09 per cent of the calculated total variance of 0.2368 is accounted for by differences between litters and 83.45 per cent is due to differences between the two breeds. The part of the variance within breeds is of the same size as in the preceding analysis. Fig. 23 shows the regressions for relative birth weights on litter size for the two breeds. The difference between the intercepts of the lines is highly significant ($P < 0.001$), but this case gives no more information about the difference between the breeds than does the analysis of

TABLE 61. Analysis of variance of relative birth weight of Large and Small race borne by crossbreds simultaneously.

Source of variation	D. F.	Mean square	Variance ratio	Components of variance	
				actual	per cent
Total	74	0.2191		0.2368	100.00
Between litter sizes	5	0.2311		(negative)	(negative)
Between litters	20	0.4509	1.12	0.0168	7.09
Between breeds	13	0.4024	17.96[a]	0.1976	83.45
Within breeds	36	0.0224		0.0224	9.46

[a] $P < 0.001$.

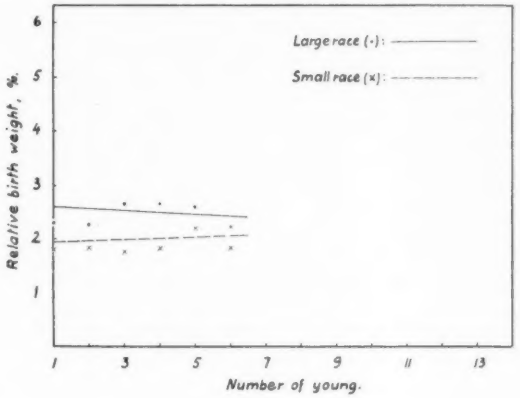


Fig. 23. Observed and estimated relative birth weight (v) in relation to litter size (x) for Large and Small race, borne by cross-breds simultaneously.

Large race: $v = 2.61 - 0.032\ x$.
Small race: $v = 1.91 + 0.025\ x$.

variance, as neither of the regression lines have significant slopes. For Polish, the coefficient of regression is, $b = 0.025 \pm 0.0268$, and for Large race, $b = -0.032 \pm 0.0281$.

DISCUSSION.

The relationship between the weight of the young and the size of the mother has been the subject of numerous investigations in which both uniparous and multiparous animals have been studied. These investigations have established that racial differences, having an indisputable genetic background, can be ascertained in the normal development of the fetuses (ECKLES 1919, MORGAN and DAVIS 1936, CASTLE 1929, KOPEĆ 1922, 1923, 1924, and many others), while at the same time it has been clearly shown that a number of factors which certainly have no genetic basis also exert a considerable modifying influence on the birth weight. Such non-genetic factors are age, or litter number and litter size (BLUHM 1929, WISHART and HAMMOND 1933, WRIGHT 1922, EATON 1932, LUSH *et al.* 1934 etc.). Most of these earlier investigations have dealt with the influence of many external factors, but in investigations into the birth weight of multiparous animals, where crosses between two breeds have been used (KOPEĆ 1922, 1923 etc., CASTLE 1929, VETULANI 1931, GREEN 1931, MARSHAK 1934, UBISH and MELLO 1940 etc.) it has not been possible to segregate the variation which is entirely due to differences in the size of the mother animals, due to the fact that normal F_1-individuals are always borne by one of the parental breeds. In consequence, the maternal influence asserts itself very strongly, as has been most clearly shown in the case of horses by WALTON and HAMMOND (1938). Even if the F_2-generation is studied this influence is still present. Failure to take into consideration the direction of the crosses in-

troduces a source of error which may interfere with the interpretation of the results. The following figures taken from investigations made at The Institute of Animal Breeding in Uppsala will illustrate the maternal after-effect from F_1 to F_2. (A detailed account of these investigations will be reported in a subsequent publication). The material, which comprises 369 young born in 64 litters of 10 different litter sizes, consists of 22 litters by small F_1-mothers, i.e. after parental crossing between Flemish Giant ♂ and Polish ♀ and 42 litters by large F_1-mothers, i.e. after crossing between Polish ♂ and Flemish Giant ♀. The average birth weights are shown in Table 62. Litter size two is represented by only one litter in each of the groups, in which case the birth weight is higher for young borne by the small mother, but for all the other litter sizes there is a large difference between the mean weights in the expected direction, a difference which is highly significant ($P < 0.005$) when calculated within litter sizes. It is therefore clear that the maternal influence is still of considerable importance in the F_2-generation.

VOL. 31 1950

However, the possibility of entirely eliminating the modifying influence of the size of the mother is small in multiparous animals. It appears that it has been achieved in only one case, viz. in the group comprising the development of Polish fetuses in Large race does. Here no decrease in the birth weight with increasing litter size has been ascertained until the litter comprises eight young, and it can therefore be asserted that in this case the maximum birth weight of Polish has been attained. This maximum has been reached in the optimum uterine environment, which has not only permitted the polygenic system to

TABLE 62. Mean birth weight of F_2-young borne by reciprocal F_1-does.

Breed of doe	SL-crossbreds		LS-crossbreds	
Litter size	Number of young	Mean birth weight, gms.	Number of young	Mean birth weight, gms.
1	—	—	—	—
2	2	48.0	2	52.5
3	18	51.3	—	—
4	24	54.1	20	44.9
5	24	52.8	30	38.3
6	48	54.1	30	40.3
7	48	50.4	14	41.0
8	24	50.1	8	45.0
9	36	43.3	9	36.4
10	10	39.7	—	—
11	11	38.1	11	32.9
Total . . .	245		124	

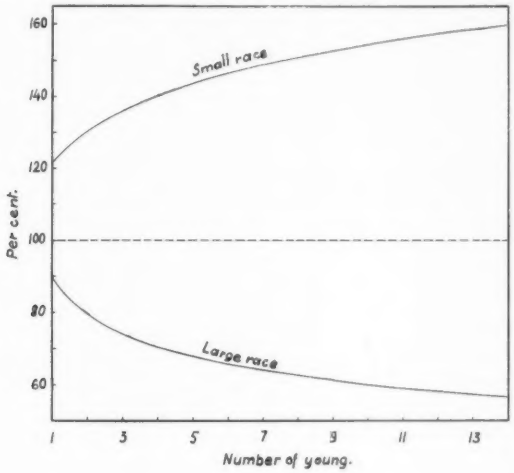


Fig. 24. Transplanted Large and Small race as a percentage of normal, based on the power function, in relation to litter size.

express itself completely, but has also allowed the interaction of different factors full play. In none of the other experimental groups have the same conditions prevailed and it is therefore not known whether the weights which have been observed for litter size one in these groups can be regarded as the maximum size of these young. In order to solve the problem it is necessary to create developmental potentialities such that the fetal weights in at least the two first litter sizes are the same.

Conversely, it might be enquired whether the development of Large race fetuses in Polish does has taken place in such a restricted uterine environment that the minimum size for this race has been reached. If this minimum size has in fact been attained it would be represented by the weight of the young born in the largest litters, but it cannot be supposed that the birth weight would remain completely constant with a further increase in the litter size. It would therefore seem desirable to adopt another criterion, and it appears reasonable to use the viability of the young as a standard. In the four litters of six there has been only one stillborn young, which means that the maximum litter size has not been reached in these investigations. A feature common to the two groups comprising transplantation of the pure breeds is the very strong modification of the birth weight. This modification can be best expressed by calculating the weight of the transplanted young as a percentage of that of normal young. In Fig. 24 this is shown for the reciprocal transplantations of pure breeds, although the calculated weights are based on the values estimated from the power functions. The use of such estimates is biologically justifiable as has already been discussed and enables more precise comparisons to be made between litter sizes, for not only are the estimates for any one litter size more precise, being based on the data for all litters, but in addition comparisons may

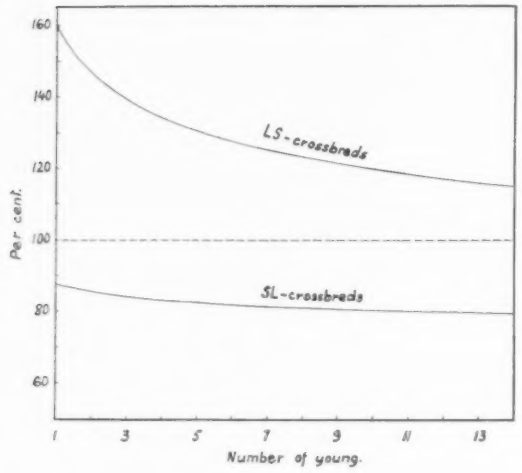


Fig. 25. Transplanted SL- and LS-crossbreds as a percentage of normal, based on the power function, in relation to litter size.

be made between litters which have not in fact been observed in the experimental material. The curves shown in the figure are apparently symmetrical, but a closer study reveals that even if the initial weights are 20 per cent above and 20 per cent below the normal weights, the increase is greater in the case of Polish than of Large race. The former shows an artificially induced modification of the birth weight of 40 per cent already in litters of four, whereas in Large race the litters must be twice as large for the reduction to amount to 40 per cent.

Fig. 25 shows the modification for the reciprocal crossbreds, calculated in similar way. In this case, however, the curves are not symmetrical. For the SL-crossbreds the form of the curve is as would be expected, the initial modification approaches 10 per cent, while in a litter of eight the modification is 20 per cent. Thus the course of the curve is entirely intermediate in relation to pure Large race. For the LS-crossbreds, the maximum modification is found in litters of one, where it amounts to 159.23 per cent. It seems anomalous that the greatest difference between normal and transplanted is to be found in the smallest litter size. On the other hand, the 20 per cent difference for a litter of eight is in complete agreement with the differences observed previously for the other groups. The explanation of this large difference must presumably be that the normal weights observed in this material are not sufficiently reliable. The consequence of this is that the calculated function does not show the true decrease in the birth weight on an increase in the number of young. The calculated function indicates that it is not the weights of the transplanted young which deviate, since it satisfactorily fits the observed data for the reciprocal, but normal, young. For the pure breeds there is either an increase, or a decrease in the birth weight depending on whether the fetuses develop in mothers

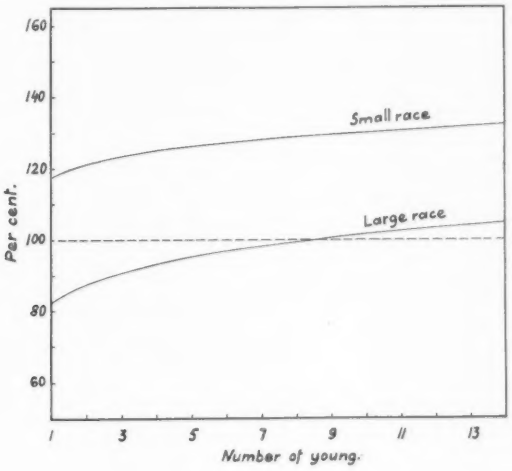
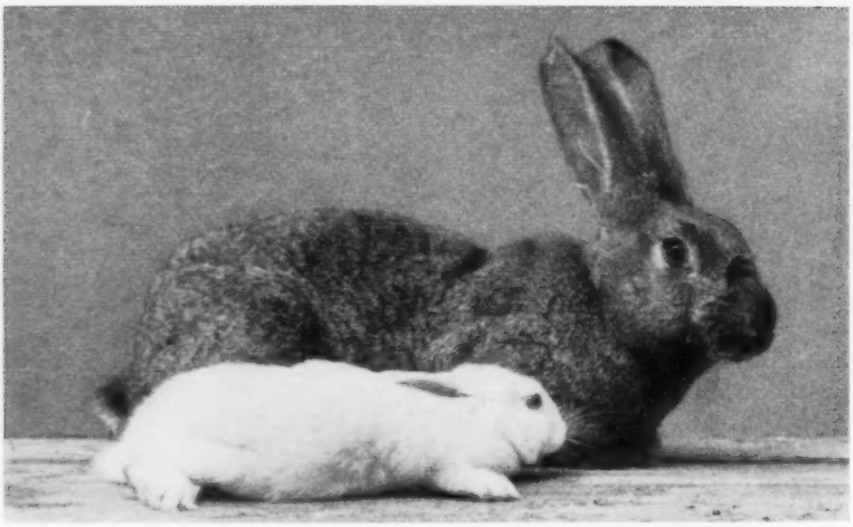
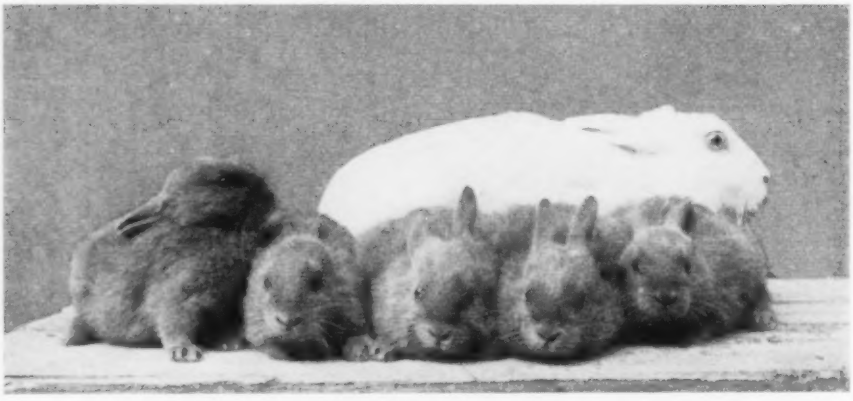


Fig. 26. Transplanted as a percentage of normal, based on the power function, in relation to litter size. Pure breeds born by crossbreds simultaneously.

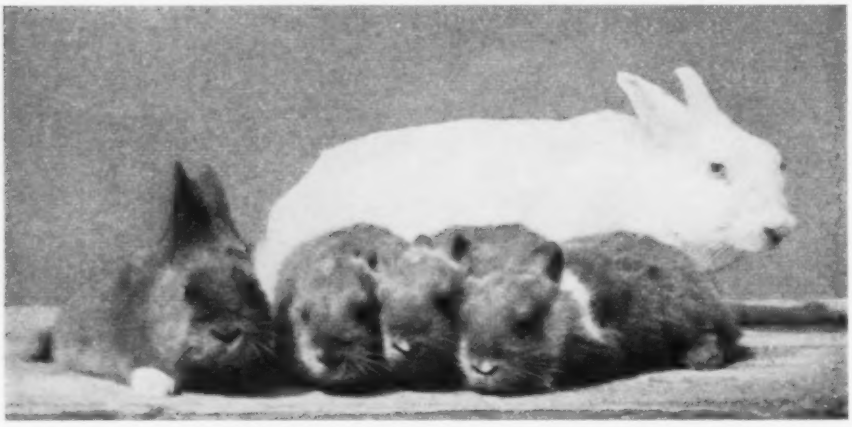
belonging to the large or small breed. This modification is least in the small litter sizes (20 per cent), but can amount to about 40 per cent in larger litters. In the case of the crossbreds the modification appears to amount to the half of these figures. For pure breeds born by crossbred does (Fig. 26), at least for the Polish breed, there is a modification which is accounted for by the greater uterine space, and which corresponds to that found in Polish borne by Large race. This modification, however, does not exceed 133 per cent of the normal weight of Polish. For Large race, likewise developed in crossbred does, the decrease in the birth weight is, however, not so great as for normal, which have a larger number of young in the litter. There is a considerable decrease in the case of singly born young, where the weight amounts to only about 80 per cent of the normal weight, and this reduction is due to the limited uterine space. A continuous decrease follows an increase in the litter size up to eight, but then disappears, for the calculated birth weight for litters of nine corresponds exactly to the normal weight. This is because the calculated weight does not in this case correspond to the weight of the normal, as the development of the Large race and Polish fetuses has taken place together. It has been observed here, as has already been referred to in the discussion of KOPEĆ's results (1922), that the observed litter size in a mixed litter actually corresponds to a "biological" litter size which is smaller for the larger race and larger for the smaller race. However, the difference between the breeds is not in all cases so clearly shown. For the pure breeds borne by crossbreds the weight of the Polish young has not in any case reached the same weight as Large race young within the same litter, but in a few mixed litters borne by mothers of the pure breeds this has been observed, as may be seen from the following summary:



a. A Flemish Giant doe with one Polish young.



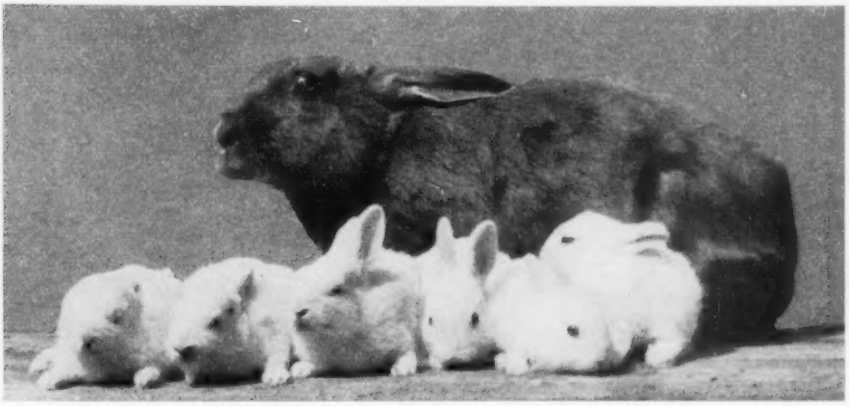
c. A Polish doe with five Blue Vienna young.



e. A Polish doe with four SL-crossbred young.

Figs. 27 a, c, e. Transplanted litters.

VOL
31
195



b. A Blue Vienna doe with six Polish young.

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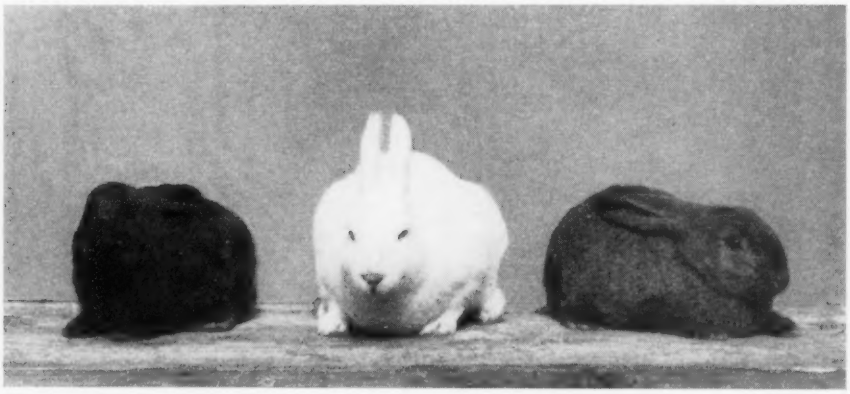
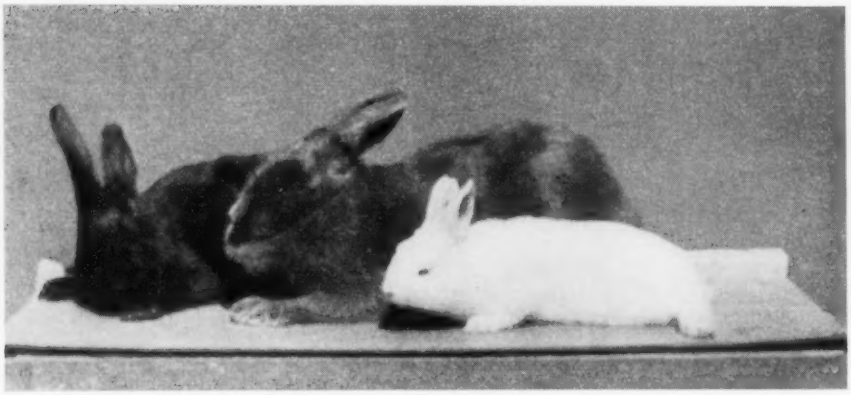


d. A Polish doe with two Flemish Giant young.



f. A SL-crossbred doe with one Polish and one Flemish Giant young.

Figs. 27 b, d, f. Transplanted litters.

Maternal breed:	Small	Small	Small	Large
Litter number:	*(S) L: 1*	*(S) L: 2*	*(S) L: 22*	*(L) S: 13*
Weight and breed	28 L	61 L	54 L	47 S
of the young;	30 L	48 S	55 S	42 S
L = Large race,	32 L	49 S		49 S
S = Small race.	27 L	51 S		43 L
	17 S			39 L
	20 S			43 L
	23 S			49 L
				40 L
				30 L
				(?) L
				(?) L

KOPEĆ (1922) supposed that the presence of fetuses of different genotypes results in a reciprocal positive influence without, however, being able to substantiate this. It would also be possible to draw this conclusion from the present investigations if consideration is restricted to the last two litters, in which the mean weight of Polish is even higher than that of Large race. In the two other litters, however, the racial differences are quite obvious, and there is nothing whatever to indicate that a "reciprocal positive influence" is exerted.

If the difference between Large race and Polish, both normally born, or both born after transplantation, is examined, the variance between the groups is seen to be represented by the sum of the variation which is partly accounted for by differences in genetic structure and partly by differences in uterine environment (Table 63). This variance is of course highly significant ($P < 0.001$) for the normal groups, amounting to 66.34 per cent of a total variance of 306.36. In the case of the transplanted groups, however, the variance does not represent more than 20.31 per cent of a calculated variance of 179.13 ($P < 0.05$). This is due to the fact that because of the transplantations a considerable decrease of the birth weight for Large race and an increase for Polish take place. The difference which can thus be ascertained is partly an expression of the extent of the interaction, but it is not possible to separate the genetic interaction from the interaction between the different environmental factors or from the interaction between heredity and environment. In the case of the crossbreds, the difference between SL and LS will in the main be an expression of differences in uterine environments. Certainly, there is a difference in the composition of the variances with regard to both the genetic and the cytoplasmic parts, but from the results previously presented the conclusion can be drawn that there is no special influence of a cytoplasmic origin on the size of the young, and it has not been possible to detect any differences in the sizes of the young which can be ascribed to a special X- or

Y-linked inheritance. The calculations on which the comparison between the reciprocal crossbreds are based (Table 63) show excellent agreement between normal and transplanted. In the one case the absolute variance is twice as great as in the other, but as relative variance the difference between the two groups amounts to 30 to 31 per cent of the total variance. By comparing these values with those stated previously, summarized in Table 63, they are seen to agree except in the case of the group $D_I + D_{II}$. The deviation of this group has previously been discussed. On the basis of the calculated variances for all the groups with regard to the differences in uterine environments it can therefore be said that the change in environment involves not only a considerable modification of the absolute birth weight, but also a radical change in the individual parts of the variance.

In Table 63 the several variances for differences between the groups have been grouped according to the differences which they reveal, i.e. whether they show uterine, genetic or cytoplasmic differences. There is no need to discuss the cytoplasmic difference in detail again, but there are no grounds for supposing that there would be any cytoplasmic difference between Large and Small race which may influence the birth weight.

The genetic difference which can be measured by these comparisons, partly as differences between the pure breeds and partly as differences between one of the pure breeds and a cross group, shows on the average that it is expressed most distinctly in a uterine environment which may be presumed to offer the fetuses comparatively poor potentialities for development. These results are in direct contrast to the results of investigations in monozygous twins in cattle (Bonnier and Hansson 1946), in which it was concluded that genetically determined differences between animals are most strongly expressed when these animals are maintained on a high plane of nutrition. There is, however, an essential difference between these two investigations. In the case of monozygous twins, the conclusions are based on differences in the extra-uterine environment (high or low plane of nutrition), whereas with rabbits, it is preponderantly the differences in the intra-uterine environment which assert themselves. It can be taken for granted that the uterine space is extremely limited for large litters of Large race, but nothing is in fact known of the quantities of nutrient which are available for each individual fetus. It is therefore not possible to distinguish between the purely mechanical and the purely physiological influences brought about by the smaller size of the uterus in Polish than in Large race.

TABLE 63. Summary of comparisons between normal and transplanted young.

I. *Variation in birth weight due to differences in uterine environment.*

Group	Breed	Variance		P
		actual	per cent	
$A_I + A_{II}$	Large race	78.70	33.70	< 0.001
$B_I + B_{II}$	Small race	46.25	41.80	< 0.001
$C_I + C_{II}$	SL-crossbreds	29.27	24.86	< 0.05
$D_I + D_{II}$	LS-crossbreds	115.64	69.05	< 0.001

II. *Variation in birth weight due to differences in cytoplasm.*

Group	Breeds compared	Variance		P
		actual	per cent	
$C_I + D_{II}$	Normal SL-crossbreds with transplanted LS-crossbreds	non	non	—
$C_{II} + D_I$	Transplanted SL-crossbreds with normal LS-crossbreds	20.56	20.78	< 0.01[1]

[1] See text.

III. *Variation in birth weight due to differences in genetic structure.*

α. Young developed in Large race does.

Group	Breeds compared	Variance		P
		actual	per cent	
$A_I + B_{II}$	Large race with Small race	11.32	6.32	—
$A_I + C_I$	Large race with SL-crossbreds	28.12	14.94	< 0.05
$A_I + D_{II}$	Large race with LS-crossbreds	5.86	3.35	—
$B_{II} + C_I$	Small race with SL-crossbreds	non	non	—
$B_{II} + D_{II}$	Small race with LS-crossbreds	8.43	7.64	—

β. Young developed in Small race does.

$A_{II} + B_I$	Large race with Small race	43.44	41.03	< 0.001
$A_{II} + C_{II}$	Large race with SL-crossbreds	2.63	1.31	—
$A_{II} + D_I$	Large race with LS-crossbreds	38.98	29.16	< 0.001
$B_I + C_{II}$	Small race with SL-crossbreds	25.78	27.59	< 0.001
$B_I + D_I$	Small race with LS-crossbreds	7.87	11.05	< 0.001

γ. Young developed in crossbred does.

E	Large race with Small race	174.39	89.02	< 0.001

IV. *Variation in birth weight due to differences in uterine environment, cytoplasm, and genetic structure.*

Group	Breeds compared	Variance		P
		actual	per cent	
$A_I + B_I$	Large race with Small race	203.24	66.34	< 0.001
$A_{II} + B_{II}$	Large race with Small race	36.38	20.31	< 0.05
$C_I + D_I$	SL-crossbreds with LS-crossbreds . . .	23.76	31.06	< 0.01
$C_{II} + D_{II}$	SL-crossbreds with LS-crossbreds . . .	44.91	30.14	< 0.01

APPENDIX.

Chemical and Histological Examinations of Stillborn Fetuses.

In the following will be given a few results of simple chemical and histological examinations of fetuses which were either stillborn or died immediately after birth. The reason that these examinations have been made is that as the length of gestation is approximately constant whereas the weigths are highly variable, it might be possible to detect differences of development between normal and transplanted fetuses. Unfortunately it has not been possible to undertake the examinations immediately after the fetuses were born, and most have been stored for some length of time in a 10 % Formalin solution. This has probably resulted in reactions in, and decompositions of skeletal parts (*e.g.* decalcification), for which it has not been possible to allow in the analyses. The chemical analyses, which have been made by the Analytical Laboratory of the Royal Agricultural College, have been the dry weight and the content of calcium, phosphorus and total ash. In addition, the dry weights of the liver and the length of the left femur have been measured (removed before determination of Ca, P, ash and dehydrated weight). A staining of the femur with Alizarin Red S and Toluidine Blue with a view to determining the ossification of the epiphyses (WILLIAMS 1941) has not given any result because of the storing in Formalin.

Table 64 shows the means for the pure breeds and transplanted SL-crossbreds, and also the ratios between the means for transplanted and normal. In the case of Polish there is an obvious difference between both the birth weights and the dry weights, and likevise for the liver weights, but if the liver weight is calculated per 100 gms. of dry weight there appears to be no difference between normal and transplanted. The ratio of calcium to phosphorus and ash, which would be the best criteria in these investigations for differences in development, shows no statistically significant differences. There is a significant difference ($P < 0.001$) in the length of femur between the two groups, but it

TABLE 64. Means for chemical composition, and length of femur, of pure breeds and SL-crossbreds.

	Number of young	Mean weight of young, gms.	Dry weight, gms.	Dry weight of liver, gms.	$\frac{\text{Dry weight of liver}}{\text{Dry weight}}$, %	Ca	$\frac{\text{Ashes}}{\text{Dry weigth}}$, %	Length of femur, cm.
Normal Large race	26	49.81	9.2096	0.9534	10.65	1.62	11.41	1.76
Transpl. Large race	29	56.07	8.9353	0.9651	11.20	1.57	10.18	1.81
$\frac{\text{Transplanted}}{\text{Normal}}$	—	1.13	0.97	1.01	1.05	0.97	0.89[2]	1.03
Normal Polish	14	34.36	6.9157	0.6005	9.39	1.53	10.61	1.64
Transpl. Polish	4	51.00	11.1725	1.0239	9.38	1.57	11.59	1.88
$\frac{\text{Transplanted}}{\text{Normal}}$	—	1.48	1.62[1]	1.71[1]	1.00	1.03	1.13	1.15[3]
SL-crossbreds, transpl.	19	56.37	10.79	0.9227	8.85	1.56	10.89	1.88

[1] $P < 0.05$; [2] $P < 0.01$; [3] $P < 0.001$.

is entirely due to the differences in size and cannot be regarded as indicating varying degrees of development. For Large race there is no difference between the mean weights of the fetuses examined, nor for any of the other characters examined. The ash content (ash/100 gms. dry weight), however, is an exception, for it is higher in normal than in transplanted fetuses. Between the two normal breeds there are clear differences for the weight of the liver and the length of the bone; there is a slightly significant difference ($P < 0.05$) in the dry weights and Ca/P, but no differences can be found between the breeds for dry weights of liver/100 gms. of dry weight or for the ash content.

For the crossbreds, information is only available for nineteen transplanted fetuses, borne by Polish. In this group, as in transplanted Large race, it is generally the largest fetuses which die at birth or are already dead at birth, and the figures are therefore not taken from a representative sample. The mean weight of these SL-fetuses was 56.4 gms., and the mean dry liver weight/100 gms. of dry weight was 8.85 gms., which latter is not significantly different from normal Polish, but on the other hand the mean for Large race has been found to be significantly higher ($P < 0.01$). GREGORY and GOSS (1933) determined the content of glutathione per 100 gms. weight and likewise found a significant difference between Flemish Giant and crossbreds between Flemish Giant and Polish.

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SUMMARY.

This study of the maternal influence on the birth weight of rabbits is based both on normal breeding experiments undertaken during the years 1938—1948, and on experiments involving transplantations of fertilized ova during the years 1946—1949. The material has comprised not only Polish rabbits as Small race, and Flemish Giant, Vienna Blue and crosses between them as Large race, but in addition, reciprocal crossbreds between Large and Small race have also been used.

1. The planning of the investigations is discussed, and a detailed description given of the technique of ova transplantation. The results of the transplantation experiments show a pregnancy percentage of 44.6 in 1946, of 53.9 in 1947, of 71.6 in 1948 and of 53.3 in 1949. The decline in 1949 is attributed to a deterioration in health of the animals. The average for all transplantations is 57.0 per cent pregnancy. Of the ova transplanted to does which have subsequently become pregnant, 48.1 per cent have developed into young. It has not been possible to show any differences between the breeds as donors or receptors.

2. The mean number of released ova per individual is 5.52 for Polish and 10.73 for Large race. There is no difference between the right and left ovaries

in reproductive capacity. A closer relationship between body weight and number of released ova is shown for Polish than for Large race. The largest number of ova found after a mating was eighteen in Large race and ten in Polish. While on the average there is no difference between the two breeds in the time when the ova have reached the two-cell stage, the cell division seems to be more rapid in Polish than in Large race if the mean division stage is made the basis of the calculation.

The importance of the albuminous layer in protecting the ovum during the storage *in vitro* has been examined. In Large race the largest number of fetuses developed from the ova whose layer was classed as "medium". In Polish on the other hand, there was no difference in the percentage development from the three classes, and it appears most probable that the albuminous layer is not of any considerable importance as a means of protection. The difficulty of making an objective visual judgement is stressed.

VOL. 31 1950

An examination of the implantation percentage clearly shows the difficulties in obtaining large litters after transplantation of fertilized ova. The length of the gestation period has been determined both for the normal pure breeds and for the transplantation groups. In all probability there is a delayed implantation of the blastocysts after transplantation, resulting in a slightly longer gestation period. A case of extra-uterine development of two fetuses has been noted.

3. The influence of a number of factors on the birth weight has been examined. Several were shown to exert an influence, but with the litter size constant, the influence of the majority of the factors was reduced. The conclusion was drawn that it is only necessary when calculating the modification of the birth weight due to transplantation to segregate the influence of the litter size. The suitability of each of three different regressions to describe the relationship between the birth weight and the litter size has been examined. This subject is discussed in detail in subsequent sections.

The variation in the birth weight within litters has been examined. While there is a difference in the absolute variation, it has not been possible to show differences in the coefficients of variation, between either breeds or litter sizes.

Probable causes of the appearance of stillborn fetuses are discussed. The principal cause of such mortality in this material is that the fetuses are too large. After development in Small race mothers 38.5 per cent of the Large race fetuses have been dead at birth, and 23.6 per cent of the SL-crossbreds have been stillborn.

4. The principles for the partitioning of the total variance is discussed. In this work it has been undertaken as follows: $\sigma^2_H + \sigma^2_C + \sigma^2_{El} + \sigma^2_{Eu} + \sigma^2_{Er} = \sigma^2_T$, H indicating the total variance attributable to genetic influences (heredity in the broad sense), C representing the cytoplasmic portion, and E the variance caused by different environmental factors, the first of these being litter size,

the second being intra-uterine environment, and the third is the influence of environment due to chance (error).

The correlations between the observed data and values calculated from the power function $y = ax^{-b}$, the exponential function $y = ab^{-x}$, and the straight line $y = a - bx$ indicate that on the whole the power function shows the best agreement. The variation in the birth weight is examined and the different parts of the variance calculated by the method developed by WINSOR and CLARKE (1940).

5. The environmental modification of the birth weight is estimated by comparing transplanted and normal young. In the case of Large race the modification amounts to 30 to 40 per cent and is greatest in the large litters. The variance for differences in uterine environments is 33.70 per cent of the total variance. In Polish the absolute modification is 20 to 40 per cent, the variance for differences in the uterine environments amounting to 41.80 per cent of the total variance. The absolute modification for SL-crossbreds is 20—30 per cent, the variance being 24.86 per cent of the total. On the average, the reciprocal crossbreds range 33 per cent above the normal weight due to development in a more favourable uterine environment. The variance for differences in uterine environments is 69.05 per cent of the total variance. On the assumption that there are no special sex-linked or cytoplasmic inheritance, comparisons between both the normal, reciprocal crossbreds and the transplanted, reciprocal crossbreds will provide a further estimate of uterine difference. The variances for the differences between the groups are 31.06 per cent of the total for normal and 30.41 per cent for transplanted.

6. The comparison between normal LS-crossbreds and transplanted SL-crossbreds or between normal SL-crossbreds and transplanted LS-crossbreds indicates the cytoplasmic effect. In the one case there is no variance, while in the other it amounts to 20.78 per cent of the total. After a detailed analysis of the data this large variance is assumed to be non-cytoplasmic, and it is concluded that there is no cytoplasmic influence on the birth weight for either of the two pure breeds.

7. The relative birth weight is calculated for all groups, and the different sources of variation are examined. The difference between the groups is determined as for the absolute birth weight, but there does not appear to be any great disagreement between the two methods. The difference between the regression lines for relative birth weight on litter size has been studied in the case of the groups in which it has been possible to keep the genetic and the cytoplasmic parts of the variance constant, so that the only difference between the groups has been the uterine environment. The relationship has been treated as linear and this regression has fitted the observed values extremely well within the range of observed litter sizes.

8. By keeping the uterine (and cytoplasmic) factor constant the variance

for genetic differences between the various groups have been calculated. The genetic difference is most fully expressed when the development of the fetus takes place in a comparatively limited uterine space.

9. An account is given of the results of a few chemical and histological examinations of stillborn fetuses.

REFERENCES.

BERNSTEIN, A. D. 1938. The participation of the corpus luteum and uterus in the implantation of the transplanted egg. Bul. de Biol. et de Med. Expt. de l'URSS, *5*, 17.

BLUHM, A. 1929. Über einige das Geburtsgewicht der Säugetiere beeinflussende Faktoren. Arch. f. Entwickl. Mech. der Organ., *116*, 348.

BONNIER, G. and HANSSON, A. 1946. Studies on monozygous cattle twins. V. The effect of different plans of nutrition on growth and body development of dairy heifers. Acta Agric. Suecana, *1*, 171.

BONNIER, G. och TEDIN, O. 1940. Biologisk variationsanalys. Stockholm.

CARMICHAEL, W. J. and RICE, J. B. 1920. Variations in farrow: with special reference to the birth weight of pigs. Ill. Agr. Expt. Sta. Bul., *226*.

CASTLE, W. E. 1909. Studies of inheritance in rabbits. Carnegie Inst. Wash. Pub., *114*.

— 1914. The nature of size factors as indicated by a study of correlation. Carnegie Inst. Wash. Pub., *196*, 51.

— 1921. On a method of estimating the number of genetic factors concerned in cases of blending inheritance. Science, *54*, 93.

— 1922. Genetic studies of rabbits and rats. I. Size-inheritance in rabbit crosses. Carnegie Inst. Wash. Pub., *320*.

— 1929. A further study of size inheritance in rabbits, with special reference to the existence of genes for size characters. Jour. Expt. Zool., *53*, 421.

— 1931. Size inheritance in rabbits; the backcross to the large parent race. Jour. Expt. Zool., *60*, 325.

— 1933. The gene theory in relation to blending inheritance. Natl. Acad. Sci. Proc., *19*, 1011.

— 1934 a. Size inheritance in rabbits; further data on the backcross to the small race. Jour. Expt. Zool., *67*, 105.

— 1934 b. Body size of reciprocal hybrids in rabbit crosses. Natl. Acad. Sci. Proc., *20*, 621.

— 1941. Size inheritance. Amer. Nat., *75*, 488.

CASTLE, W. E. and GREGORY, P. W. 1929. The embryological basis of size inheritance in the rabbit. Jour. Morph. and Physiol., *48*, 81.

CASTLE, W. E., GATES, W. H., and REED, S. C. 1936 a. Studies of a size cross in mice. Genetics, *21*, 66.

CASTLE, W. E., GATES, W. H., REED, S. C., and LAW, L. W. 1936 b. Studies of a size cross in mice. Genetics, *21*, 310.

CHANG, M. C. 1948. The effects of low temperature on fertilized rabbit ova in vitro, and the normal development of ova kept at low temperature for several days. Jour. Gen. Physiol., *31*, 385.

CHAPMAN, A. B. and LUSH, J. L. 1932. Twinning, sex ratios and genetic variability in birth weight in sheep. Jour. Hered., *23*, 473.

CLARK, R. T. 1934. Studies on the physiology of reproduction in the sheep. Anat. Rec., *60*, 135.

VOL. 31 1950

DETLEFSEN, J. A. 1914. Genetic studies on a cavy species cross. Carnegie Inst. Wash. Pub., *205*.

EATON, O. N. 1932. Correlation of hereditary and other factors affecting growth in guinea pigs. U. S. Dept. Agr. Tech. Bul., *279*.

ECKLES, C. H. 1919. A study of birth weight of calves. Mo. Agr. Expt. Sta. Res. Bul., *35*.

ENZMANN, E. V. and CROZIER, W. J. 1935. Relation between birth weight and litter size in multiparous mammals. Jour. Gen. Physiol., *18*, 791.

FELDMAN, H. W. 1935. The brown variation and growth in the house mouse. Amer. Nat., *69*, 370.

FITCH, J. B., MCGILLIARD, P. G., and DRUMM, G. M. 1924. A study of the birth weight, and gestation of dairy animals. Jour. Dairy Sci., *7*, *222*.

GATES, W. H. 1925. Litter size, birth weight, and early growth rate of mice (Mus musculus). Anat. Rec., *29*, 183.

GOWEN, J. W., STADLER, J., and JOHNSON, L. E. 1946. On the mechanism of heterosis—the chromosal or cytoplasmic basis for heterosis in Drosophila melanogaster. Amer. Nat., *80*, 506.

GREEN, C. V. 1930. Stillbirths in a mouse interspecific cross (Mus musculus × Mus bactrianus). Soc. Expt. Biol. and Med. Proc., *28*, 55.

— 1931. Size inheritance and growth in a mouse species cross (Mus musculus × Mus bactrianus). II. Birth weights. Jour. Expt. Zool., *58*, 247.

— 1935 a. Quantitative characters in reciprocal hybrids. Amer. Nat., *69*, 278.

— 1935 b. Apparent changes with age in crossing-over between colour and size genes in mice. Jour. Genet., *30*, 101.

GREEN, W. W. and WINTERS, L. M. 1945. Prenatal development of the sheep. Minn. Agr. Expt. Sta. Tech. Bul., *169*.

GREENE, H. S. N. 1940. A dwarf mutation in the rabbit. The constitutional influence on homozygous and heterozygous individuals. Jour. Expt. Med., *71*, 839.

GREENE, H. S. N., HU, C. K., and BROWN, W. H. 1934. A lethal dwarf mutation in the rabbit with stigmata of endocrine abnormality. Science, *79*, 487.

GREGORY, P. W. 1930. The early embryology of the rabbit. Carnegie Inst. Wash. Pub., *407*, 141.

— 1932. The potential and actual fecundity of some breeds of rabbits. Jour. Expt. Zool., *62*, 271.

GREGORY, P. W. and CASTLE, W. E. 1931. Further studies on the embryological basis of size inheritance in the rabbit. Jour. Expt. Zool., *59*, 199.

GREGORY, P. W. and GOSS, H. 1933. The relation of sulphydryl concentration to size inheritance in the rabbit. Amer. Nat., *67*, 180.

GRÜNEBERG, H. 1943. The genetics of the mouse. Cambridge.

HAINES, G. 1931. A statistical study of the relation between various expressions of fertility and vigor in the guinea pig. Jour. Agr. Res., *42*, 123.

HALD, S. 1948. Statistiske Metoder m. Tabel- og Formelsamling. København.

HALL, V. E. and PIERCE, G. N. 1934. Litter size, birth weight and growth to weaning in the cat. Anat. Rec., *60*, 111.

HAMMOND, J. 1921. Further observations on the factors controlling fertility and foetal atrophy. Jour. Agr. Sci., *11*, 337.

— 1934. The fertilisation of rabbit ova in relation to time. A method of controlling the litter size, the duration of pregnancy and the weight of the young at birth. Jour. Expt. Biol., *11*, 140.

— 1935. The changes in the reproductive organs of the rabbit during pregnancy. Trudy po Din. Rasv. [Moskva], *10*, 93.

HAMMOND, J. and MARSHALL, F. H. 1925. Reproduction in the rabbit. Edinburgh.

HANSSON, A. 1945. Dräktighetstidens inverkan på kornas levande vikt. K. Lantbr. Akad. Tidskr., *84*, 368.

HARTMAN, C. G., LEWIS, W. H., MILLER, F. W., and SWETT, W. W. 1931. First findings of tubal ova in the cow, together with notes on estrus. Anat. Rec., *48*, 267.

HEAPE, W. 1890. Preliminary note on the transplantation and growth of mammalian ova within uterine foster-mother. Roy. Soc. London, Proc., *48*, 457.

— 1897. Further note on the transplantation and growth of mammalian ova within uterine foster-mother. Roy. Soc. London, Proc., *62*, 178.

HUXLEY, J. S. 1932. Problems of relative growth. London.

IBSEN, H. L. 1928. Prenatal growth in guinea-pigs with special reference to environmental factors affecting weight at birth. Jour. Expt. Zool., *51*, 51.

KEELER, C. E. 1947. The effect upon ultimate body size produced by combining certain coat character genes in the Norway rat. (Tenn. Acad. Sci. Jour., *22*, 99), Anim. Breeding Abs. 1948, *16*, nr. 1486.

KEILMANN, A. 1891. Zur Klärung der Cervixfrage. Ztschr. Geburtsh. und Gynäk., *22*, 106.

KINCAID, C. M. 1943. Influence of the sire on the birth weight of lambs. Jour. Anim. Sci., *2*, 152.

KING, H. D. 1915. On the weight of the albino rat at birth and the factors that influence it. Anat. Rec., *9*, 213.

KNAPP, B., LAMBERT, W. V., and BLACK, W. H. 1941. Factors influencing length of gestation and birth weight in cattle. Jour. Agr. Res., *61*, 277.

KOPEĆ, S. 1922. (On the offspring of two sires in one litter of rabbits.) Inst. Natl. Polon. Écon. Rurale Pulawy, Mém., *2*, 214.

— 1923 a. (On the inheritance of weight in rabbits from a study of the newborn.) Inst. Natl. Polon. Écon. Rurale Pulawy, Mém., *4*, 218.

— 1923 b. On the offspring of rabbit-does mated with two sires simultaneously. Jour. Genet., *13*, 371.

— 1923 c. (The influence of certain developmental factors on the birth weight of mammals.) Inst. Natl. Polon. Écon. Rurale Pulawy, Mém., *4*, 173.

— 1924 a. On the influence exerted by certain inheritance factors on the birth weight of rabbits. Anat. Rec., *27*, 95.

— 1924 b. Studies on the inheritance of weight of new-born rabbits. Jour. Genet., *14*, 241.

— 1925. (On the correlation between the weight of new-born, and that of growing and full-grown rabbits.) Inst. Natl. Polon. Écon. Rurale Pulawy, Mém., *6*, 288.

— 1926. The morphological value of the weight of rabbits at birth. Jour. Genet., *17*, 187.

KREIDL, A. und NEUMANN, A. 1911. Über eine gesetzmässige Abhängigkeit der Grössenverhältnisse der Föten vom Orte der Anheftung im Uterus bei multiparen Tieren. Akad. der Wiss. Wien, Math.-Nat. Kl., Sitzber. Abt. *3*, 141.

LAW, L. W. 1938. Studies on size inheritance in mice. Genetics, *23*, 309.

LAWRENCE, P. S. 1941. The sex ratio, fertility, and ancestral longevity. Quart. Rev. Biol., *16*, 35.

LONG, J. F., GERLAUGH, P., and RIFE, D. C. 1948. A genetic study of gestation period in cattle. Rec. Genet. Soc. Amer., *17*, 46.

LUSH, J. L. 1943. Animal breeding plans. Ames, Iowa.

— 1949. Quantitative characters. Eighth Internatl. Cong. Genet. Proc., Hereditas, 356.

LUSH, J. L., HETZER, H. O., and CULBERTSON, C. C. 1934. Factors affecting birth weights of swine. Genetics, *19*, 329.

MACARTHUR, J. W. 1949. Selection for small and large body size in the house mouse. Genetics, *34*, 194.

MACDOWELL, E. C. 1914. Size inheritance in rabbits. Carnegie Inst. Wash. Pub., *196*, 1.

MANRESA, M. 1933. Physiology of reproduction in the rabbit. Phillipine Jour. Sci., *51*, 323.

VOL. 31 1950

MARSHAK, A. 1934. Inheritance of growth rates in mice. Unpublished.
— 1936. Growth differences in reciprocal hybrids and cytoplasmic influence on growth in mice. Jour. Expt. Zool., *72*, 497.
MATHER, K. 1949. Biometrical genetics. London.
MCKENZIE, F. F. 1928. Growth and reproduction in swine. Miss. Agr. Expt. Sta. Res. Bul., *118*.
MENSHOW, B. 1935. (Live weight at birth as a basis of selection.) Problemy Zhivotnovodstva, *9*, 70.
MINOT, C. S. 1891. Senescence and rejuvenation. Jour. Physiol., *12*, 97.
MORGAN, R. F. and DAVIS, H. P. 1936. The effect of pregnancy and parturition on the weight of dairy cows. Nebr. Agr. Expt. Sta. Res. Bul., *82*.
MOSELEY, T. W., STUART, D., and GRAVES, R. R. 1929. Dairy work at the Huntley field station, Huntley, Mont. 1918—1927. U. S. Dept. Agr. Tech. Bul., *116*.
MURRAY, W. S. 1934. The breeding behaviour of the dilute brown stock of mice (Little dba). Amer. Jour. Cancer, *20*, 573.
OLOFSSON, N. E. och LARSSON, S. 1930. Undersökningar angående modersuggornas produktion och smågrisarnas utveckling. K. Lantbr. Akad. Handl. och Tidskr., *69*, 559.
OLSEN, H. 1939. Pattegrisenes Levedygtighed og Vækstevne. Landøkonom. Forsøgslab. Beret. *186*, 125.
PAIM, H. D. 1944. Estudo sôbre a duração do periodo de gestação e o pêso ao nascer plantél Hereford do pôsto zootécnico da serra. Rev. de Agr. [Piracicaba], *19*, 333.
PAINTER, T. S. 1928. Cell size and body size in rabbits. Jour. Expt. Zool., *50*, 441.
PEASE, M. S. 1928. Experiments on the inheritance of weight in rabbit. Jour. Genet., *20*, 261.
PICKARD, J. N. 1930. A preliminary study of some of the factors influencing the duration of pregnancy and litter size in the rabbit. Fourth World's Poultry Cong., Sect. F, 901.
PINCUS, G. 1930. Observations on the living eggs of the rabbit. Roy. Soc. London, Proc., Ser. B, *107*, 132.
— 1939 a. The comparative behaviour of mammalian eggs in vivo and in vitro. II. The activation of tubal eggs of the rabbit. Jour. Expt. Zool., *73*, 195.
— 1939 b. The comparative behaviour of mammalian eggs in vivo and in vitro. IV. The development of fertilized and artificially activated rabbit eggs. Jour. Expt. Zool., *82*, 85.
— 1939 c. The breeding of some rabbits produced by recipients of artificially activated ova. Natl. Acad. Sci. Proc., *25*, 557.
— 1940 a. The developmental physiology of rabbit eggs. N. Y. Acad. Sci., Trans., Ser. II, *3*, 6.
— 1940 b. Superovulation in rabbits. Anat. Rec., *77*, 1.
PINCUS, G. and ENZMANN, E. V. 1932. Fertilization in the rabbit. Jour. Expt. Biol., *9*, 403.
— — 1934. Can mammalian eggs undergo normal development in vitro? Natl. Acad. Sci., Proc., *20*, 121.
PUNNETT, R. C. and BAILEY, P. G. 1918. Genetic studies in rabbits. I. On the inheritance of weight. Jour. Genet., *8*, 1.
ROSAHN, P. D. and GREENE, H. S. N. 1936. The influence of intrauterine factors on the fetal weight of rabbits. Jour. Expt. Med., *63*, 901.
ROSAHN, P. D., GREENE, H. S. N., and HU, C. K. 1935. Observations on the gestation period of the rabbit. Jour. Expt. Zool., *72*, 195.
SIMPSON, S. 1913. The rate of growth in the dog. Soc. Expt. Biol. and Med. Proc., *10*, 97.
SNEDECOR, G. W. 1946. Statistical methods. Ames, Iowa.
SONNEBAR, T. M. 1948. Genes and cytoplasm in cellular heredity. Science, *108*, 580.

TYLER, W. J., CHAPMAN, A. B., and DICKERSON, G. E. 1947. Sources of variation in the birth weight of Holstein-Friesian calves. Jour. Dairy Sci., *30*, 483.

UBISCH, G. VON, and MELLO, R. F. 1940. Genetic studies on a cavy species cross. Jour. Hered., *31*, 389.

VENGE, O. 1948. Forskellige faktorers indvirkning på fødselsvægten af kalve. Nord. Jordbrugsforsk., *7—8*, 208.

VETULANI, T. 1931. Versuche über Vererbung des Gewichtes und einiger Körpermasse neugeborener Mäuse. Biol. Gen., *8*, 369.

WALTON, A. and HAMMOND, J. 1928. Observation on ovulation in the rabbit. Brit. Jour. Expt. Biol., *6*, 190.

— — 1938. The maternal effects on growth and conformation in Shire horse-Shetland pony crosses. Roy. Soc. London, Proc., Ser. B., *125*, 311.

WATERMAN, A. J. 1943. Studies of normal development of the New Zealand White strain of rabbit. Amer. Jour. Anat., *72*, 473.

WILLIAMS, T. W. 1941. Alizarin red S and toluidine blue for differentiating adult or embryonic bone and cartilage. Stain Technol., *16*, 23.

WILLIAMS, W. L. 1943. The diseases of the genital organs of domestic animals. Ithaca.

WINSOR, C. P. and CLARKE, G. L. 1940. A statistical study of variation in the catch of plankton nets. Jour. Mar. Res., *3*, 1.

WINTERS, L. M., GREEN, W. W., and COMSTOCK, R. E. 1942. Prenatal development of the bovine. Minn. Agr. Expt. Sta. Tech. Bul., *151*.

WISHART, J. and HAMMOND, J. 1933. A statistical analysis of the interrelations of litter size and duration of pregnancy on the birth weight of rabbit. Jour. Agr. Res., *23*, 463.

WRIGHT, S. 1918. On the nature of size factors. Genetics, *3*, 367.

— 1921. Correlation and causation. Jour. Agr. Res., *20*, 557.

— 1922. The effects of inbreeding and crossbreeding on guinea pigs. III. Crosses between highly inbred families. U. S. Dept. Agr., Bul., *1121*.

— 1932. General, group and special factors. Genetics, *17*, 603.

— 1935. The analysis of variance and the correlation between relatives with respect to deviations from optimum. Jour. Genet., *30*, 243.

YULE, G. U. and KENDALL, M. G. 1948. An introduction to the theory of statistics. London.

VOL. 31 1950

DETLEFSEN, J. A. 1914. Genetic studies on a cavy species cross. Carnegie Inst. Wash. Pub., *205*.

EATON, O. N. 1932. Correlation of hereditary and other factors affecting growth in guinea pigs. U. S. Dept. Agr. Tech. Bul., *279*.

ECKLES, C. H. 1919. A study of birth weight of calves. Mo. Agr. Expt. Sta. Res. Bul., *35*.

ENZMANN, E. V. and CROZIER, W. J. 1935. Relation between birth weight and litter size in multiparous mammals. Jour. Gen. Physiol., *18*, 791.

FELDMAN, H. W. 1935. The brown variation and growth in the house mouse. Amer. Nat., *69*, 370.

FITCH, J. B., MCGILLIARD, P. G., and DRUMM, G. M. 1924. A study of the birth weight, and gestation of dairy animals. Jour. Dairy Sci., *7*, *222*.

GATES, W. H. 1925. Litter size, birth weight, and early growth rate of mice (Mus musculus). Anat. Rec., *29*, 183.

GOWEN, J. W., STADLER, J., and JOHNSON, L. E. 1946. On the mechanism of heterosis—the chromosal or cytoplasmic basis for heterosis in Drosophila melanogaster. Amer. Nat., *80*, 506.

GREEN, C. V. 1930. Stillbirths in a mouse interspecific cross (Mus musculus × Mus bactrianus). Soc. Expt. Biol. and Med. Proc., *28*, 55.

— 1931. Size inheritance and growth in a mouse species cross (Mus musculus × Mus bactrianus). II. Birth weights. Jour. Expt. Zool., *58*, 247.

— 1935 a. Quantitative characters in reciprocal hybrids. Amer. Nat., *69*, 278.

— 1935 b. Apparent changes with age in crossing-over between colour and size genes in mice. Jour. Genet., *30*, 101.

GREEN, W. W. and WINTERS, L. M. 1945. Prenatal development of the sheep. Minn. Agr. Expt. Sta. Tech. Bul., *169*.

GREENE, H. S. N. 1940. A dwarf mutation in the rabbit. The constitutional influence on homozygous and heterozygous individuals. Jour. Expt. Med., *71*, 839.

GREENE, H. S. N., HU, C. K., and BROWN, W. H. 1934. A lethal dwarf mutation in the rabbit with stigmata of endocrine abnormality. Science, *79*, 487.

GREGORY, P. W. 1930. The early embryology of the rabbit. Carnegie Inst. Wash. Pub., *407*, 141.

— 1932. The potential and actual fecundity of some breeds of rabbits. Jour. Expt. Zool., *62*, 271.

GREGORY, P. W. and CASTLE, W. E. 1931. Further studies on the embryological basis of size inheritance in the rabbit. Jour. Expt. Zool., *59*, 199.

GREGORY, P. W. and GOSS, H. 1933. The relation of sulphydryl concentration to size inheritance in the rabbit. Amer. Nat., *67*, 180.

GRÜNEBERG, H. 1943. The genetics of the mouse. Cambridge.

HAINES, G. 1931. A statistical study of the relation between various expressions of fertility and vigor in the guinea pig. Jour. Agr. Res., *42*, 123.

HALD, S. 1948. Statistiske Metoder m. Tabel- og Formelsamling. København.

HALL, V. E. and PIERCE, G. N. 1934. Litter size, birth weight and growth to weaning in the cat. Anat. Rec., *60*, 111.

HAMMOND, J. 1921. Further observations on the factors controlling fertility and foetal atrophy. Jour. Agr. Sci., *11*, 337.

— 1934. The fertilisation of rabbit ova in relation to time. A method of controlling the litter size, the duration of pregnancy and the weight of the young at birth. Jour. Expt. Biol., *11*, 140.

— 1935. The changes in the reproductive organs of the rabbit during pregnancy. Trudy po Din. Rasv. [Moskva], *10*, 93.

HAMMOND, J. and MARSHALL, F. H. 1925. Reproduction in the rabbit. Edinburgh.

HANSSON, A. 1945. Dräktighetstidens inverkan på kornas levande vikt. K. Lantbr. Akad. Tidskr., *84*, 368.

HARTMAN, C. G., LEWIS, W. H., MILLER, F. W., and SWETT, W. W. 1931. First findings of tubal ova in the cow, together with notes on estrus. Anat. Rec., *48*, 267.

HEAPE, W. 1890. Preliminary note on the transplantation and growth of mammalian ova within uterine foster-mother. Roy. Soc. London, Proc., *48*, 457.

— 1897. Further note on the transplantation and growth of mammalian ova within uterine foster-mother. Roy. Soc. London, Proc., *62*, 178.

HUXLEY, J. S. 1932. Problems of relative growth. London.

IBSEN, H. L. 1928. Prenatal growth in guinea-pigs with special reference to environmental factors affecting weight at birth. Jour. Expt. Zool., *51*, 51.

KEELER, C. E. 1947. The effect upon ultimate body size produced by combining certain coat character genes in the Norway rat. (Tenn. Acad. Sci. Jour., *22*, 99), Anim. Breeding Abs. 1948, *16*, nr. 1486.

KEILMANN, A. 1891. Zur Klärung der Cervixfrage. Ztschr. Geburtsh. und Gynäk., *22*, 106.

KINCAID, C. M. 1943. Influence of the sire on the birth weight of lambs. Jour. Anim. Sci., *2*, 152.

KING, H. D. 1915. On the weight of the albino rat at birth and the factors that influence it. Anat. Rec., *9*, 213.

KNAPP, B., LAMBERT, W. V., and BLACK, W. H. 1941. Factors influencing length of gestation and birth weight in cattle. Jour. Agr. Res., *61*, 277.

KOPEĆ, S. 1922. (On the offspring of two sires in one litter of rabbits.) Inst. Natl. Polon. Écon. Rurale Pulawy, Mém., *2*, 214.

— 1923 a. (On the inheritance of weight in rabbits from a study of the newborn.) Inst. Natl. Polon. Écon. Rurale Pulawy, Mém., *4*, 218.

— 1923 b. On the offspring of rabbit-does mated with two sires simultaneously. Jour. Genet., *13*, 371.

— 1923 c. (The influence of certain developmental factors on the birth weight of mammals.) Inst. Natl. Polon. Écon. Rurale Pulawy, Mém., *4*, 173.

— 1924 a. On the influence exerted by certain inheritance factors on the birth weight of rabbits. Anat. Rec., *27*, 95.

— 1924 b. Studies on the inheritance of weight of new-born rabbits. Jour. Genet., *14*, 241.

— 1925. (On the correlation between the weight of new-born, and that of growing and full-grown rabbits.) Inst. Natl. Polon. Écon. Rurale Pulawy, Mém., *6*, 288.

— 1926. The morphological value of the weight of rabbits at birth. Jour. Genet., *17*, 187.

KREIDL, A. und NEUMANN, A. 1911. Über eine gesetzmässige Abhängigkeit der Grössenverhältnisse der Föten vom Orte der Anheftung im Uterus bei multiparen Tieren. Akad. der Wiss. Wien, Math.-Nat. Kl., Sitzber. Abt. *3*, 141.

LAW, L. W. 1938. Studies on size inheritance in mice. Genetics, *23*, 399.

LAWRENCE, P. S. 1941. The sex ratio, fertility, and ancestral longevity. Quart. Rev. Biol., *16*, 35.

LONG, J. F., GERLAUGH, P., and RIFE, D. C. 1948. A genetic study of gestation period in cattle. Rec. Genet. Soc. Amer., *17*, 46.

LUSH, J. L. 1943. Animal breeding plans. Ames, Iowa.

— 1949. Quantitative characters. Eighth Internatl. Cong. Genet. Proc., Hereditas, 356.

LUSH, J. L., HETZER, H. O., and CULBERTSON, C. C. 1934. Factors affecting birth weights of swine. Genetics, *19*, 329.

MACARTHUR, J. W. 1949. Selection for small and large body size in the house mouse. Genetics, *34*, 194.

MACDOWELL, E. C. 1914. Size inheritance in rabbits. Carnegie Inst. Wash. Pub., *196*, 1.

MANRESA, M. 1933. Physiology of reproduction in the rabbit. Phillipine Jour. Sci., *51*, 323.

VOL. 31 1950

MARSHAK, A. 1934. Inheritance of growth rates in mice. Unpublished.
— 1936. Growth differences in reciprocal hybrids and cytoplasmic influence on growth in mice. Jour. Expt. Zool., *72*, 497.
MATHER, K. 1949. Biometrical genetics. London.
MCKENZIE, F. F. 1928. Growth and reproduction in swine. Miss. Agr. Expt. Sta. Res. Bul., *118*.
MENSHOW, B. 1935. (Live weight at birth as a basis of selection.) Problemy Zhivotnovodstva, *9*, 70.
MINOT, C. S. 1891. Senescence and rejuvenation. Jour. Physiol., *12*, 97.
MORGAN, R. F. and DAVIS, H. P. 1936. The effect of pregnancy and parturition on the weight of dairy cows. Nebr. Agr. Expt. Sta. Res. Bul., *82*.
MOSELEY, T. W., STUART, D., and GRAVES, R. R. 1929. Dairy work at the Huntley field station, Huntley, Mont. 1918—1927. U. S. Dept. Agr. Tech. Bul., *116*.
MURRAY, W. S. 1934. The breeding behaviour of the dilute brown stock of mice (Little dba). Amer. Jour. Cancer, *20*, 573.
OLOFSSON, N. E. och LARSSON, S. 1930. Undersökningar angående modersuggornas produktion och smågrisarnas utveckling. K. Lantbr. Akad. Handl. och Tidskr., *69*, 559.
OLSEN, H. 1939. Pattegrisenes Levedygtighed og Vækstevne. Landøkonom. Forsøgslab. Beret. *186*, 125.
PAIM, H. D. 1944. Estudo sôbre a duração do periodo de gestação e o pêso ao nascer plantél Hereford do pôsto zootécnico da serra. Rev. de Agr. [Piracicaba], *19*, 333.
PAINTER, T. S. 1928. Cell size and body size in rabbits. Jour. Expt. Zool., *50*, 441.
PEASE, M. S. 1928. Experiments on the inheritance of weight in rabbit. Jour. Genet., *20*, 261.
PICKARD, J. N. 1930. A preliminary study of some of the factors influencing the duration of pregnancy and litter size in the rabbit. Fourth World's Poultry Cong., Sect. F, 901.
PINCUS, G. 1930. Observations on the living eggs of the rabbit. Roy. Soc. London, Proc., Ser. B, *107*, 132.
— 1939 a. The comparative behaviour of mammalian eggs in vivo and in vitro. II. The activation of tubal eggs of the rabbit. Jour. Expt. Zool., *73*, 195.
— 1939 b. The comparative behaviour of mammalian eggs in vivo and in vitro. IV. The development of fertilized and artificially activated rabbit eggs. Jour. Expt. Zool., *82*, 85.
— 1939 c. The breeding of some rabbits produced by recipients of artificially activated ova. Natl. Acad. Sci. Proc., *25*, 557.
— 1940 a. The developmental physiology of rabbit eggs. N. Y. Acad. Sci., Trans., Ser. II, *3*, 6.
— 1940 b. Superovulation in rabbits. Anat. Rec., *77*, 1.
PINCUS, G. and ENZMANN, E. V. 1932. Fertilization in the rabbit. Jour. Expt. Biol., *9*, 403.
— — 1934. Can mammalian eggs undergo normal development in vitro? Natl. Acad. Sci., Proc., *20*, 121.
PUNNETT, R. C. and BAILEY, P. G. 1918. Genetic studies in rabbits. I. On the inheritance of weight. Jour. Genet., *8*, 1.
ROSAHN, P. D. and GREENE, H. S. N. 1936. The influence of intrauterine factors on the fetal weight of rabbits. Jour. Expt. Med., *63*, 901.
ROSAHN, P. D., GREENE, H. S. N., and HU, C. K. 1935. Observations on the gestation period of the rabbit. Jour. Expt. Zool., *72*, 195.
SIMPSON, S. 1913. The rate of growth in the dog. Soc. Expt. Biol. and Med. Proc., *10*, 97.
SNEDECOR, G. W. 1946. Statistical methods. Ames, Iowa.
SONNEBAR, T. M. 1948. Genes and cytoplasm in cellular heredity. Science, *108*, 580.

TYLER, W. J., CHAPMAN, A. B., and DICKERSON, G. E. 1947. Sources of variation in the birth weight of Holstein-Friesian calves. Jour. Dairy Sci., *30*, 483.

UBISCH, G. VON, and MELLO, R. F. 1940. Genetic studies on a cavy species cross. Jour. Hered., *31*, 389.

VENGE, O. 1948. Forskellige faktorers indvirkning på fødselsvægten af kalve. Nord. Jordbrugsforsk., *7—8*, 208.

VETULANI, T. 1931. Versuche über Vererbung des Gewichtes und einiger Körpermasse neugeborener Mäuse. Biol. Gen., *8*, 369.

WALTON, A. and HAMMOND, J. 1928. Observation on ovulation in the rabbit. Brit. Jour. Expt. Biol., *6*, 190.

— — 1938. The maternal effects on growth and conformation in Shire horse-Shetland pony crosses. Roy. Soc. London, Proc., Ser. B., *125*, 311.

WATERMAN, A. J. 1943. Studies of normal development of the New Zealand White strain of rabbit. Amer. Jour. Anat., *72*, 473.

WILLIAMS, T. W. 1941. Alizarin red S and toluidine blue for differentiating adult or embryonic bone and cartilage. Stain Technol., *16*, 23.

WILLIAMS, W. L. 1943. The diseases of the genital organs of domestic animals. Ithaca.

WINSOR, C. P. and CLARKE, G. L. 1940. A statistical study of variation in the catch of plankton nets. Jour. Mar. Res., *3*, 1.

WINTERS, L. M., GREEN, W. W., and COMSTOCK, R. E. 1942. Prenatal development of the bovine. Minn. Agr. Expt. Sta. Tech. Bul., *151*.

WISHART, J. and HAMMOND, J. 1933. A statistical analysis of the interrelations of litter size and duration of pregnancy on the birth weight of rabbit. Jour. Agr. Res., *23*, 463.

WRIGHT, S. 1918. On the nature of size factors. Genetics, *3*, 367.

— 1921. Correlation and causation. Jour. Agr. Res., *20*, 557.

— 1922. The effects of inbreeding and crossbreeding on guinea pigs. III. Crosses between highly inbred families. U. S. Dept. Agr., Bul., *1121*.

— 1932. General, group and special factors. Genetics, *17*, 603.

— 1935. The analysis of variance and the correlation between relatives with respect to deviations from optimum. Jour. Genet., *30*, 243.

YULE, G. U. and KENDALL, M. G. 1948. An introduction to the theory of statistics. London.

VOL.
31
1950

ON THE NERVOUS SYSTEM OF GLOSSOBALANUS MARGINATUS MEEK (ENTEROPNEUSTA)

BY

LARS SILÉN

CONTENTS.

1. Introduction 149
2. Extension of the nervous layers 151
a. Epidermis 151
b. Intestine 152
c. Coelom 154
3. Components of the nervous layers 158
a. Sense cells 158
b. Ganglion cells and nerve fibres 162
4. Summary and discussion 170
Literature 174
Abbreviations 175

1. INTRODUCTION.

The structure of the nervous system of the Enteropneusts has long remained rather superficially known. As late as 1945 BULLOCK was in a position to characterize the standpoint in this matter thus: "Virtually nothing is known of the system except the gross anatomic facts — — —". Further researches have thus seemed desirable.

The present research was performed on the two forms *Glossobalanus marginatus* Meek and *Protoglossus* n. sp., both of them collected in the vicinity of the marine zoological station Kristineberg on the west coast of Sweden. It is a pleasure to acknowledge my great debt to the director of that institute, Dr. GUNNAR GUSTAFSON, who made this work possible by procuring for me, in the summer of 1948, 8 specimens of *Glossobalanus* and about 70 of *Protoglossus*.

In the present area these animals live in comparatively deep water and are necessarily collected by means of dredging. Owing to this fact all the specimens collected are, unfortunately, more or less incomplete, the hind parts of their fragile trunk regions being crushed or torn off by the too rough treatment.

Consequently, the present author has not been able to examine the parts of the nervous system proximal to the liver region, parts that, it is however true, are of a secondary interest only.

Part of the material was fixed according to such general methods as those of SMITH, ZENKER, etc., and then was stained with HEIDENHAIN'S azan or HEIDENHAIN'S iron hematoxylin.

Of the previous examinators of the Enteropneusts only HESS and BULLOCK have tried specific nerve stains with any success. HESS (1937, 1938) treated body portions with methylene blue or according to BODIAN'S protargol impregnation method and then usually mounted them *in toto*. However, his technical results are not easily judged. The histological description given by him has been severely criticized by BULLOCK (1945) and, indeed, seems questionable for fundamental reasons (cf. further below). BULLOCK (1944, 1945) used the same dyes on living specimens then crushed and on sectioned material respectively. He got certain definite results. By the former method some types of neurons became apparent though not their position and connections. The BODIAN impregnation was not complete nor very selective. Thus, for instance, the sense cells did not appear though undoubtedly present.

As the staining of the nervous system of certain invertebrate groups, among them the Enteropneusts, indeed affords many special difficulties, a survey of the methods tried by the present author may not wholly lack interest. They represent, so far, variations of the BODIAN method exclusively, experiments with methylene blue and with PALMGREN'S (1948) silver staining method having as yet met with no success.

Part of the animals were narcotized by means of magnesium sulphate, cocain, or 5 per cent alcohol, of which the two first are generally recommended in the literature. Part of them were narcotized by means of an addition by degrees to the sea water in which they had been placed of drops of the fixation fluid later used. They were then fixed by means of the following fluids tried by BODIAN (ROMEIS 1948, § 1817): Nos. 1 (sea water substituted for the 0.9 per cent sodium chloride solution), 2, 3, 5, 6, 7, 9 (ANCONA), 13 (BOUIN). They were immediately washed and then dehydrated, cleared, and embedded in paraffin as rapidly as possible, this with regard to, *i.a.*, the reputedly bad influence of alcohol on the tissue of the Enteropneusts.

The optimal thickness of the sections was found to be 6—8 μ. The use of so thin sections is, obviously, a drawback in nerve researches. However, it was a necessity as the minute and entangled components of the nervous system of these animals could not be distinguished in thicker sections.

The subsequent staining according to BODIAN met with little or no success after most of the above treatments. However, comparatively good results were attained in animals narcotized with BOUIN'S fluid—which proved to be excellent for that purpose, quite superior to the usual narcotics—and fixed in the same

VOL. 31 1950

fluid. There the nervous system was stained with a considerable degree of selectivity. However, variations appeared in different parts of the body. Superficially situated parts were, generally, more feebly stained than deeply situated ones. Though some theoretical uncertainty remains in so far as one cannot, of course, say definitely what structures have n o t been stained, the coherent picture of the nervous system obtained indicates, nevertheless, that probably no features of importance have remained altogether unstained.

The investigation has been carried out in the Zootomical Institute of the University of Stockholm. I am indebted to Fil. kand. R. OLSSON at that institute for his valuable aid in performing the sectioning and staining work.

The present paper is mainly based on the conditions in *Glossobalanus*. Already in this large species the nervous components are minute and in the small *Protoglossus* species they proved to be too inconspicuous to be usable for more than the confirmation, to a limited extent, of observations made in *Glossobalanus*. Mainly such results are advanced which, with regard to the considerable anatomical uniformity of the Enteropneusts, can be expected to be of general validity.

As VAN DER HORST (1927—1939) and BULLOCK (op. cit.) have recently given excellent and exhaustive surveys of the previous work on the nervous system of the Enteropneusts, a comprehensive survey in this place is superfluous.

2. EXTENSION OF THE NERVOUS LAYERS.

a. *Epidermis.*

The nervous system of the Enteropneusts has an intraepithelial position between the basal parts of the epithelial cells close to the basal membrane (limiting membrane or boundary zone) as is well-known. Its chief part is situated in the epidermis, where it, largely, forms a coherent layer round the entire body Along the dorsal and ventral median lines of the trunk it is thickened into cords. The distal end of the ventral one is joined to the dorsal one by semicircular continuations at the distal border of the trunk. As has been demonstrated by BULLOCK (1945) the ventral cord is considerably stronger than the large part of the dorsal one situated in the same region. The dorsal cord passes distally into the invaginated nerve-cord of the collar region ("Kragenmark"), which is continued as a superficial cord through the proboscis stalk into a latero-ventral circular thickening of the proboscis nerve-layer near the emanation point of the stalk.

One or two additional remarks can be made about the general distribution of the nervous system in the epidermis.

VAN DER HORST (1927, p. 39.) states that the nerve layer of the trunk is extremely thin in regions with a low epithelium. BULLOCK (1945, pp. 82—83)

says that in *Saccoglossus* "The glandular islands" of the sides of the abdomen "show a well defined nerve-fibre layer" and "represent, in fact, the only places in the lateral epithelium where nervous tissue is demonstrable, in ordinary sections" but that in *Ptychodera* there are "no extensive non-glandular areas of the abdomen. The epithelium and its nerve layer are well developed right back to the anal end". It is not quite clear to me if BULLOCK uses the word abdomen in the same strict sense as VAN DER HORST (op. cit., p. 23) or if he means by this term the entire trunk. According to BULLOCK "over the gonads the epithelium may become thin and the nerve layer disappear", apparently in the Enteropneusts in general.

In the two forms examined by me the nerve layer of the trunk is quite distinct and, largely, of equal thickness everywhere between the dorsal and ventral cords notwithstanding the variations of the epithelial structure, thus also above the gonads. However, the conditions of the proximal part of the trunk are unknown to the writer.

Of course, it is not possible to estimate with exactitude to what degree, in comparison with previous descriptions, this more complete picture of the extension of the epidermal nervous layer depends on specific differences. However, it will be seen with full evidence below that other details of the nervous system as well appear more completely than according to previous accounts. Thus there are reasons for thinking that this relative completeness is mainly due to greater success in the staining, which indicates a general validity of the observations.

BULLOCK mentions (op. cit., pp. 73 and 75) that he has failed to find any nervous tissue in the walls of the coelomoducts of the proboscis or collar. However, in *Glossobalanus* a nerve layer is quite distinct in both places (fig. 1). The epithelium of the exterior parts of the ducts is ciliated and contrasts with their non-ciliated interior parts. It is generally agreed that the former is of ectodermal, the latter of mesodermal origin. The nerve layer is comparatively thick near the pores but then gradually decreases inwards and disappears completely at the inner limit of the ciliated epithelium. Thus its extension here coincides with the extension of the ectoderm. In spite of a most careful scrutiny no nervous elements were found to enter the coelom this way.—The ciliated epithelium of the exterior part of a proboscis duct and the epidermis in the same area are situated so near each other that they have a basal membrane in common. Consequently, their respective nerve layers are separated by that membrane only.

b. *Intestine.*

According to the general opinion the intestine is of entodermal origin throughout its entire extension.

The occurrence of a nervous stratum in the intestinal epithelium has been

VOL
31
195

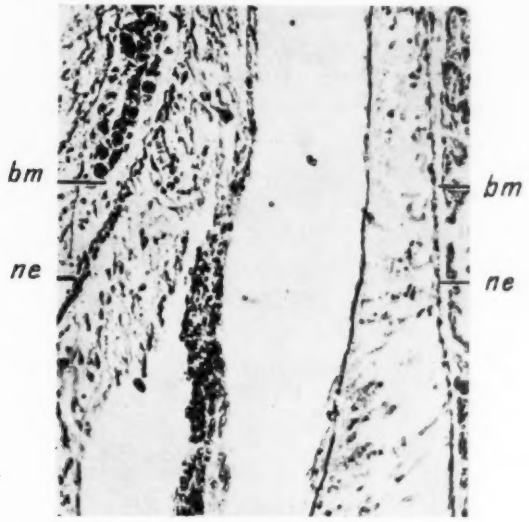

Fig. 1. Longitudinal section of part of proboscis duct, showing nervous layer. 350 ×. For letters, see p. 175.

demonstrated by some previous authors. Our present knowledge of its extension can be summed up as follows (BULLOCK 1945, pp. 83 and 87). The nerve layer of the epidermis, when reaching the mouth, is directly continued by a well-developed layer in the buccal epithelium. It "is frequently thickened in the mid-dorsal and occasionally in the mid-ventral line" and is said to extend into the pharynx, "where it disappears except in the epibranchial and hypobranchial stripes". In certain forms it has been observed in the pharyngeal faces of the gills but in no species on their anterior and posterior faces. In the remaining part of the alimentary canal nervous tissue has not been observed except in the terminal intestine of such forms as *Balanoglossus*.—Nerve-fibres are the only components distinguished in the layer.

In *Protoglossus* hardly more is distinctly seen than a well developed buccal layer extending into the pharynx. In the other parts of the gut scattered fragments of a nerve layer have been stained in my slides.

In *Glossobalanus* the writer has succeeded in getting a far more complete picture of this tissue.

Here a coherent nervous stratum surrounds the entire gut in all the regions represented in this material, *i.e.* the buccal cavity, pharynx, oesophagus, and the "stomach" back towards the liver region (figs. 2—10). However, in the intestinal diverticulum of the proboscis, the "notochord", special conditions reign, to which we shall return below.

Round the oral opening the nervous layer is approximately of the same

thickness as in the epidermis in general. The nervous layer of the gut extending from there is distinctly thinner and decreases slowly in thickness back towards the oesophagus. Here it consists of one or two fibre layers only. This thickness is then constant further back into the liver region (fig. 2) and it may thus be surmised that the nerve layer continues along the proximal part of the gut, which is missing in my material.

Along the mid-ventral line of the buccal cavity, pharynx, and oesophagus there runs a considerably thickened nerve cord by degrees getting thinner towards its sides and passing into the thin general layer (figs. 4—6). Though more inconspicuous further proximally it can be followed backwards through the whole extension of my preparates. A similar but feebler cord appears along the mid-dorsal line (fig. 3). The cords pass into the thick layer surrounding the mouth.—These cords correspond, in all probability, to the thickenings along the buccal cavity reported by BULLOCK and what are apparently their continuations "in the epibranchial and hypobranchial stripes".

Nervous tissue is present in all the epithelia of the gills as well as of the gill septa, thus also in their posterior and anterior faces. In the pharyngeal faces it is represented by a comparatively thick layer containing, *i.a.*, numerous fibres running circularly in relation to the longitudinal axis of the animal. In the other epithelia it is developed as a simple, one-layered, equally disposed plexus.

Nerve cell bodies, not only in the shape of "ganglion cells" but also of sense cells, are present in the intestinal nervous tissue (cf. below).

The coherent nerve layer of the buccal cavity epithelium ceases almost abruptly where the latter passes into the epithelium of the proboscis diverticulum. Only single and scattered fibres that are with certainty of a nervous nature have been observed in the latter and then not far from its mouth. The BODIAN impregnation has made a great number of peculiar cells appear throughout the entire diverticulum epithelium. Though they have some general resemblance to sense cells, they are probably of a non-nervous nature (cf. below).

This description of the gross structure of the intestinal nervous layer reveals a remarkable similarity to the conditions in the epidermis of the trunk. A strong ventral and a feebler dorsal cord contrast with a, largely, uniformly thin, coherent nervous layer.

c. *Coelom.*

There has been much deliberation about what nervous elements exists between the basal membranes of the epidermis and the gut, particularly in respect to the problem of the innervation of the muscles. It has been clear from fundamental reasons that such elements must be present there but only few and

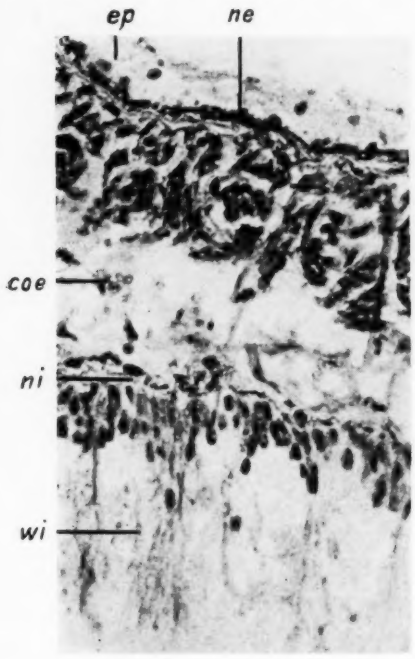

Fig. 2.

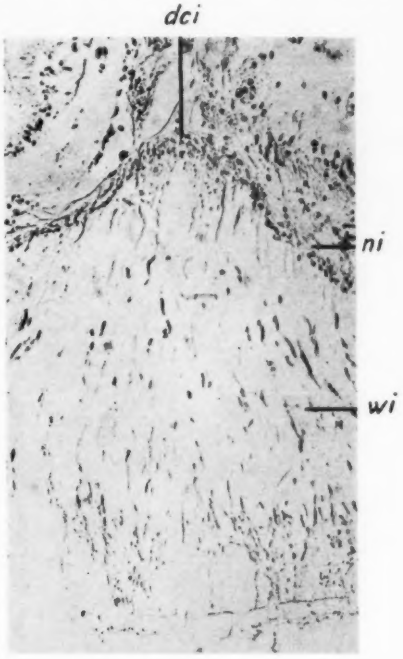

Fig. 3.

Fig. 2. Cross section of low epidermis and intestinal wall with nervous layers, in lateral part of genital region. 400 ×.
Fig. 3. Cross section of dorsal wall of pharynx with dorsal intestinal nerve cord. 390 ×.
For letters, see p. 175.

uncertain observations have been made of possibly nervous structures. VAN DER HORST (1927, p. 39) states: "— — —, von der Innervierung der Muskeln wissen wir noch gar nichts ".

In *Glossobalanus sarniensis* SPENGEL (1893) found two longitudinal thin cords extending in each side of the dorsal blood vessel which were apparently composed of "Punktsubstanz"; the surrounding cells had some resemblance to nerve cells. He did not observe any connections with the nervous layer of the epidermis or with other tissues or organs. In *Glossobalanus elongatus* the same author (1904) was not able to detect any similar structures. BULLOCK (1945, p. 88) has indistinctly seen structures "suggestive of parts of SPENGEL's description" in *Balanoglossus occidentalis* and *Glossobalanus sp.* but in no other species.

HESS (1937, p. 168) briefly mentions that "A multipolar nerve network was observed in certain internal regions of the body" and gives a diagrammatic picture of it. In his opinion it supplies the muscle fibres. This picture is reminiscent of the idea cautiously advanced by VAN DER HORST (1927, p. 39):

"Ich halte es für möglich, dass die reich verästelten 'Bindegewebszellen', welche zwischen den Muskeln liegen, motorische Nervenzellen sind." BULLOCK (op. cit., pp. 87, 89) has also touched upon this problem and found it dubious.

Finally, there is the question of the nerve connections which, from fundamental grounds, must be supposed to penetrate the basal membranes. In the proboscis BULLOCK (op. cit., pp. 65—66, fig. 20) has on one occasion observed fibres traversing the basal membrane of the epidermis, the further course of which he was unable to follow. He cautiously pointed out the possibility that these structures are nerve fibres and that the organs of the coelom may be supplied by short nerve fibres diffusely crossing the epidermal basal membrane everywhere.

It can be noted that the innervation of the vascular system is wholly unknown.

In *Glossobalanus marginatus* the present author has found a well developed "internal nervous system" along the v e n t r a l mesentery within the part of the body represented in the present material. It is particularly distinct in a cross section series from the proximal part of the genital region (figs. 4—6). It consists of coherent plexuses extending along the entire exterior side of the double mesentery (*i.e.* the sides turned to the coelomic cavities) as well as below a broad strip of the ventral basal membrane (*i.e.* below the epidermis) in each side of the ventro-median fixation-line of the mesentery. They are composed of fibres as well as of nerve cell bodies. They lie immediately at the respective membranes, thus holding the same position in relation to the latter as the nerve layers of the epidermis and gut to their respective basal membranes. The plexuses on the two sides of the mesentery are not completely separated but the mesentery is densely perforated by nerve fibres above and below the ventral blood vessel it surrounds.

Numerous fibres are seen to pierce the basal membranes of the epidermis and the gut on each side of the respective fixation lines of the mesentery, thus connecting the ventral cords of the epidermal and intestinal nervous layers with the coelomic plexuses (fig. 6).

From the plexuses fibres extend at about right angles, inward towards the lumen of the ventral blood vessel and outward towards the body cavity. The former end abruptly at the same level, possibly in the thin muscle layer known to surround the vessel; the latter extend for a short distance and terminate among the muscles of the coelom. In no case have I been able to observe connections between these fibres and the numerous, distinctly stained "connective cells".

It ought to be emphasized that no structures of a distinctly nervous nature were found along the dorsal mesentery. In this connection the fact is possibly of some significance that the ventral nerve cords of the epidermis as well as of the gut—that is, the cords that are undoubtedly and intimately connected

VOL.
31
195

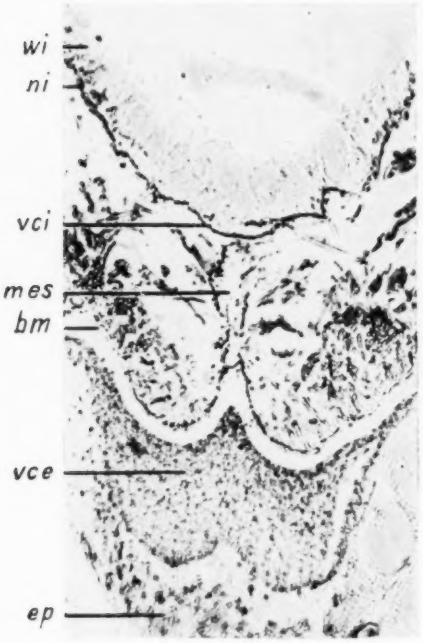

Fig. 4.

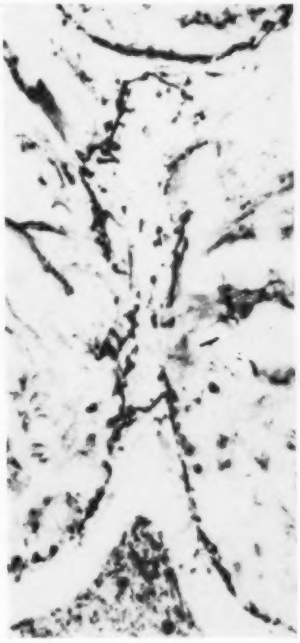

Fig. 5.

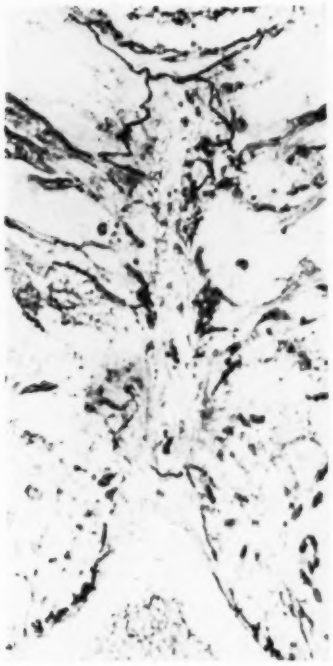

Fig. 6.

Nervous system in coelom.

Fig. 4. Cross section of ventral part of genital region, showing ventral cord of epidermis, coelomic nerve plexus along ventral mesentery, and intestinal nerve layer with ventral cord. Artificial slits between epidermal cord and basal membrane, and in intestinal cord. 160 ×.

Fig. 5. The same section, coelomic plexus. 400 ×.

Fig. 6. Another section of the same structures, showing connections between coelomic plexus and cords. 400 ×.

For letters, see p. 175.

with the nervous layer of the coelom—are considerably stronger than their dorsal counterparts.

The present author has not succeeded in observing structures that could with certainty be interpreted as nerve fibres penetrating the basal membranes outside the vicinity of the ventral mesentery.

3. COMPONENTS OF THE NERVOUS LAYERS.

a. *Sense cells.*

There has been rather a lot of uncertainty as to what type of sense cells the Enteropneusts could be expected to possess as they have escaped direct observation until quite recently. BATESON (1886) considered all of the ciliated epidermis cells to be sense cells. However, it has been clear to most authors that only certain epidermal cells ought to be sense cells and, more explicitly, primary sense cells. This theoretical conclusion has been illustrated by such diagrammatic pictures of the epidermal structure with sense cells inset as those advanced by VAN DER HORST (1939, p. 648) and DAWYDOFF (1948, p. 380).

BULLOCK (1945, p. 68, Pl. 8) was the first to demonstrate the sense cells with certainty by staining isolated cells with methylene blue in crushed body parts. In sections he could not distinguish them but tried to conclude about their position and number by means of counting, within a certain area of the epidermis, the nuclei, the indifferent epithelial cells, and the gland cells; the number of nuclei always proved to be greater than the total number of these cells; the latter number having been subtracted from the former one, the rest represented, in his opinion, nuclei of sense cells. Starting from the number thus arrived at he thought it possible to conclude about the characteristics of the nuclei of just the sense cells and thus further on their position.

In BODIAN preparates of *Glossobalanus marginatus* sense cells appear distinctly (figs. 7—10). The nucleus appears as an oblong—though not particularly narrow or "cigar-shaped"— body, 6—7 μ of length, always stained a pale violet and of a homogeneous structure (fig. 10). The cell usually reaches its widest diameter at the level of the nucleus. It tapers more or less rapidly in the distal direction and then passes into a more or less narrow, equilinear part reaching

Sense cells.

Fig. 7. Sense cells in pharyngeal face of gill, showing their extension from nervous layer to ciliated surface of epithelium. 700 ×.

Fig. 8. Short sense cell from anterior face of gill. Its terminal disc with central projection indicated. 800 ×.

Fig. 9. Very long sense cell in alimentary part of pharynx. 350 ×.

Fig. 10. Sense cells in pharyngeal face of gill. Left: nuclei focused; right: centrifugal fibres focused. 800 ×.

VOL. 31 1950

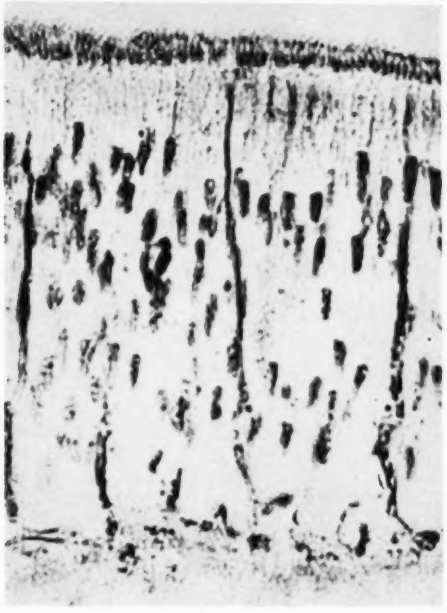

Fig. 7.

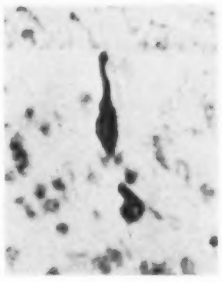

Fig. 8.

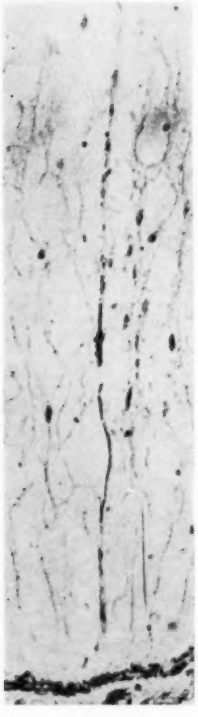

Fig. 9

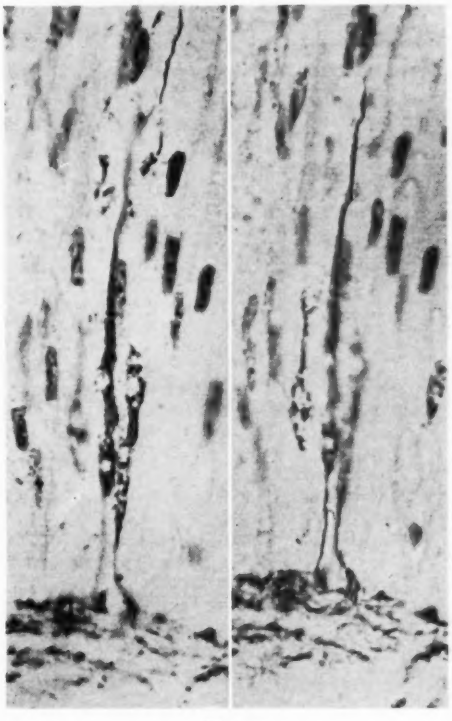

Fig. 10.

the surface of the epithelium. Having pierced the cuticle its extremity is often seen to dilate into a flat disc the centre of which is raised into a short, curved process (figs. 8, 21). Proximally to the nucleus the cell usually passes rather abruptly into a narrow fibre extending straight down to the basiepithelial nervous layer (fig. 10). The extremity of the undivided fibre passes, in all clearly observed cases, into a distinct body about 5 μ in diameter. Three or four fibres emanate from that body. Most of them extend horizontally intermingling with the other fibres of the nervous layer. Very often, however, one or two fibres are distinctly seen to extend perpendicularly to the interior part of the nervous layer.

The colour appears, in not too darkly stained cells, as unequally distributed in the cell body out of the nucleus, dark threads contrasting with lighter interspaces. They are probably comparable with the fibrillae shown in BULLOCK's methylene blue pictures of sense cells.

The length and thickness of the sense cells as well as the relative measurements of their distal, central, and proximal parts vary within wide limits. The position of the nucleus varies from about midway between the nervous layer and the surface of the epithelium to only some tens of μ outside the former.

The variations in shape can be connected with the height of the respective epithelia. The higher the epithelium, the longer and narrower are the sense cells, and the reverse. The shortest one (about 25 μ in length, fig. 8) are met with in the anterior faces of the gills, the longest ones (about 300 μ in length, fig. 9) in the hind part of the proboscis and certain parts of the epidermis of the collar and trunk as well as in the wall of the alimentary part of the pharynx. The fixation process has very possibly had a considerable influence on the picture of the sense cells appearing in the preparates, certain parts of the animals evidently being more contracted than others. However, it is of course difficult to estimate the extent of this influence.

The structure of the sense cell as described here coincides rather well, fundamentally, with those described by BULLOCK (op. cit., figs. 22, 23, 25) from methylene blue preparates. They are primary sense cells of only one morphologic type.

According to VAN DER HORST's theoretical picture of sense cells as well as BULLOCK's description, the interior end of the centrifugal fibre of the cell splits up directly into branches intertwining with the fibres of the nervous layer. This is undoubtedly what is to be expected for fundamental reasons. The large and well delimited body situated in this place in the present preparates may seem rather puzzling. The idea might even occur that it represents a nerve cell body. However, the fact that I have failed to observe a nucleus in it indicates, against the above background, that it is an artefact.

HESS (1938) has diagrammed a sense cell of about the same type. However, he lays stress above all on the fact that he has found bipolar nerve cells which

VOL. 31 1950

he designates as photoreceptors. BULLOCK has pointed out the questionable character of the entire description and denies the possibility of their being sense cells. However, it may be possible that HESS has really observed sense cells but of the type described here considering certain morphological similarities as well as their, according to his description, large size quite surpassing that of other nerve cells, though he has misinterpreted their position and detailed structure. Such misinterpretations could obviously be quite easily made in the type of total preparations that he used. However this may be, the neuron pictures in question have certainly no resemblance to other neurons observed by the present author.

BRAMBELL & COLE (1939, p. 185, fig. 4) described, in the "preoral ciliary organ" detected by them and thought to be a sense organ, cells which in their opinion were probably sense cells. According to BULLOCK (1945, p. 91) they are "almost certainly" gland cells. I must agree with him, being unable to find any important features in common with the sense cells described here. On the contrary, they greatly resemble the long and narrow granular gland cells described by VAN DER HORST (1927, fig. 26) in *Dolichoglossus*.

In *Glossobalanus marginatus* sense cells appear in most parts of the epidermis, in the interior of the collar cord directed to the small lumina of that structure, and in the epithelia of the pharyngeal faces of the gills as well as, curiously enough, in the alimentary part of the pharynx (the present species is one of the Enteropneusts in which the pharynx is incompletely divided by longitudinal lateral constrictions into a dorsal respiratory and a ventral alimentary part) and of the remaining gut backwards to the liver region at least. They are wholly absent only in some extremely low epithelia such as in parts of the anterior and posterior faces of the gills and certain lateral areas of the epidermis.

As to the frequency of sense cells per square unit I have not tried to make an estimate. In my opinion such an attempt is premature considering the fact that the sense cells are often seen to be very fragmentarily stained and we must presume that an unknown number of cells have not been stained at all. Further, the different influence of the impregnation in different areas has to be taken into consideration. Unfortunately, I have failed to discern any reliable characteristics in which the nuclei of the sense cells definitely differ from other nuclei of the epithelia, no special "cigar-shape" as reported by BULLOCK, or the like. Very generally expressed it can be stated that particularly numerous sense cells are found in the hind part of the proboscis, in the areas before and behind the collar cord, and, to a lesser degree, in the pharynx as a whole. However comparatively numerous these cells may be in certain areas they seem to be very far from reaching such a number BULLOCK arrived at by means of his counting method related above, when he states (p. 104) that "the sensory cells greatly predominate, often outnumbering all other epithelial celltypes combined".

Recent authors have failed to find any real sense organs, if we disregard the enigmatic organ described by BRAMBELL & COLE and some other structures of undecided nature, and I have not met with greater success.

In the proboscis diverticulum of the gut of *Glossobalanus* a peculiar type of cells appeared in BODIAN preparates—and there exclusively—where they were stained a dark violet (fig. 11). They are long and narrow, reaching from the basal membrane to the surface of the epithelium. Their basal parts are usually divided into two or three processes resting on the basal membrane with dilated foot plates. Their contents are of a homogeneous appearance. These cells are so densely placed throughout the whole of the diverticulum that their basal feet touch each other and form a coherent layer in wide areas.

As far as I know, no elements of this type have been discerned in the proboscis diverticulum before, nor in any other part of the body. Their general shape and position as well as the fact that they appear just in the BODIAN preparates, might indicate that they represent some sort of sense cells. However, certain facts render this interpretation improbable. The fact that their colour differs distinctly from the bluish black of undoubtedly nervous elements in immediately neighbouring areas, may be of minor importance. However, the details of their structure, such as the structure of their basal parts, hardly coincide with what is to be expected in a nerve cell. In spite of a careful search I have failed to trace any nerve fibres to or between the cells though there are plenty of them immediately outside the limit of the diverticulum. On a few occasions a small drop was observed of apparently the same character as the contents of the cell immediately outside the superficial end of the latter. This fact possibly indicates that the present cells are glands.

(Single gland cells of common type have been found in the proboscis diverticulum by previous authors (cf. VAN DER HORST 1927). However, the presence and great number of the peculiar cells now in question, apparently absent in other parts of the gut, indicate that the diverticulum possibly has a particular and important function as part of the alimentary system instead of or in addition to the function of a supporting structure previously ascribed to it. This, of course, can only be valid in species where the diverticulum is continuous with the rest of the gut.)

b. *Ganglion cells and nerve fibres.*

The remaining types of nerve cell bodies may conveniently—though perhaps not wholly adequately—be called ganglion cells in accordance with BULLOCK (1945, p. 69).

Most previous descriptions of ganglion cells in the Enteropneusts are vague. It can be generally stated, without exaggeration, that their existence has been

VOL. 31 1950

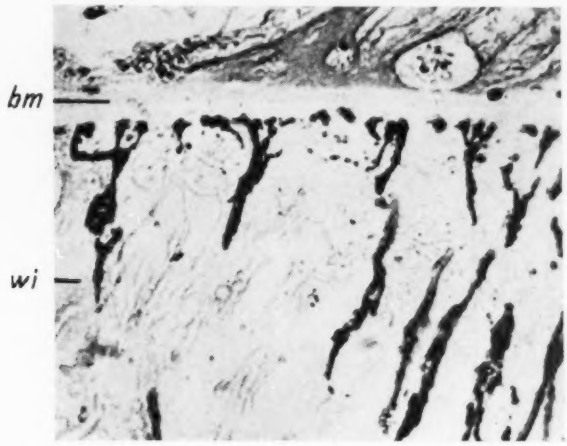

Fig. 11. Gland (?) cells in dorsal wall of proboscis diverticulum of gut. 800 ×. For letters, see p. 175.

more surmised than really observed. Only BULLOCK (op. cit., p. 69, Pl. 8) has given exact pictures of isolated cells from his methylene blue preparates.

The following quotations from BULLOCK's work may serve as a summing up of the present, partly hypothetical standpoint with regard to the position and structure of the ganglion cells. "They are all lodged in that zone of the epithelium immediately peripheral to the nerve-fibre layer — — —, that is, they are not scattered in the plexus but sharply segregated from it. Occasional exceptions are found — — —" (in the peduncle). "At present no distinction can be made between intermediate and motor neurons." "Ganglion cells of bipolar, multipolar, and unipolar varieties occur, but the last named are confined to the collar nerve-cord and adjacent levels of other nerve-cords. Bipolars are the most common, — — —." "A very few multipolar neurons were found" in the abdomen. The so-called giant cells detected by SPENGEL (1893) and lodged in the collar and dorsal trunk cords have been described by BULLOCK (1944) as unipolar motor cells.

As to the nerve fibres, BULLOCK (1945) has made the following statements. "All nerve-fibres, except the giant fibres, appear to be alike." A dichotomous and relatively infrequent branching occurs. "No differentiations of axon and dendrite, — — —, have taken place. Nor have boutons terminaux or other specialized endings been seen." The fibres are horizontal. There is no stratification within the fibre layers.

The observations of the present writer differ in several respects from these statements.

The simplest nervous layers are found in especially low epithelia, for in-

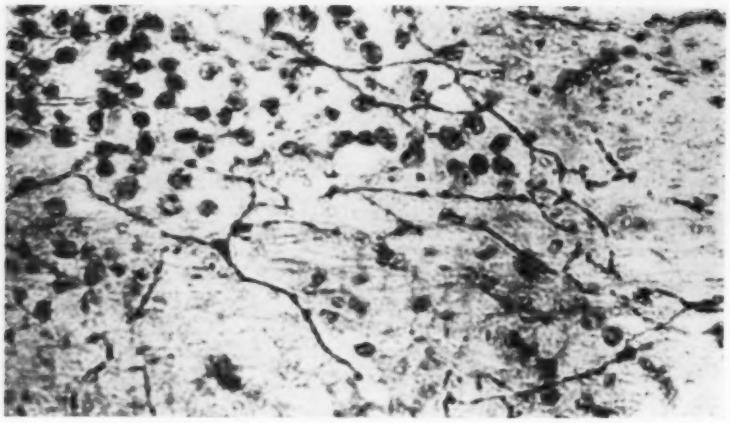

Fig. 12.

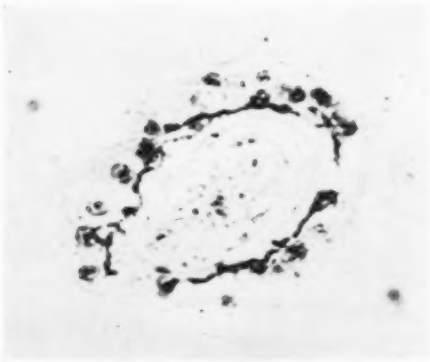

Fig. 13.

Plexus of multipolar neurons.

Fig. 12. Horizontal section through basal part of low gill epithelium, showing nerve-net with multipolar cell bodies. 700 ×.

Fig. 13. Cross section of synapticula with nerve-net. 700 ×.

stance in the anterior and posterior faces of the gills and gill septa. They are represented by one-layered nerve-nets (figs. 12, 13). In well stained sections the relatively few nerve cell bodies are easily distinguished from the numerous nuclei of indifferent epithelial cells lying so close at the plexus that the nerve fibres wind in and out between them. Their bodies are small—this is the case for all ganglion cells, except the giant cells, observed by me in the Enteropneusts—averaging 7—8 μ of diameter and often only slightly surpassing the nuclei of the indifferent cells in size. All of the distinctly observed cells are multi- (3- or 4-) polar (figs. 12, 14). The nerve processes are all alike as they seem to be in the entire nervous system (now disregarding the giant fibres).

VOL. 31 1950

Different types of ganglion cells.

Fig. 14. Multipolar cells.

Fig. 15. Bipolar cell.

Fig. 16. Ganglion cells from coelom.

They bifurcate frequently. Artefacts easily confused, at a superficial glance, with cell bodies often appear at the bifurcations. The fibres extend irregularly in all directions and form a dense network. The fact that the fibres of the different neurons appear as coherent in the present preparates does not necessarily mean, of course, that there is a protoplasmatic continuity. For theoretical reasons the reverse is more probable. The effect of the staining method has been too rough in the present case for discerning real facts to shed further light on this question.

The nervous layer of the other epithelia outside the cords is considerably thicker. The chief constituent is an entangled plexus of the type of multipolar neurons just described, their cell bodies situated at varying levels but particularly numerous near the basal membrane. Two additional components can be discerned here. The centrifugal fibres of the sense cells, present in these epithelia, divide here into horizontal and vertical fibres as has been described above. Finally, horizontal fibres occur which extend straight for long distances without bifurcating and which emanate from distinctly bipolar cells (figs. 15, 18). The cells are comparatively long, averaging 12 μ in length, but narrow and invariably maintain a horizontal position. The entire neurons can be concluded to be very long as the interspaces between the cell bodies are always considerable and a fibre can often be traced for a long distance. In certain parts of the body these fibres contrast with those of the multipolar neurons in their regular, parallel courses. They are of a distinctly longitudinal direction in the dorsal and ventral cords of the epidermis and intestinal wall. In the circular connective of the epidermis at the border between collar and trunk and in the gill septa they have a circular extension in relation to the longitudinal axis of the animal (cf. above p. 154). In the remaining nerve layers no uniform courses have been distinguished with certainty by the present author.

In a nerve cord the same elements can be identified. However, two strata are seen there (figs. 17, 18). A narrow stratum along the basal membrane corresponds in thickness and structure with the plexus of the surrounding area just described. The upper and far thicker stratum is exclusively composed of longitudinally arranged bipolar neurons. The limit between the two strata is not quite distinct but there is a transitional zone.—The extra thickness of a cord, if compared with the nervous layer in general, is thus attained by an enormous increase in the number of bipolar neurons all of them superposed on the plexiform stratum. This is further shown by the extension of the centrifugal fibres of the sense cells (figs. 18, 21). These fibres always divide at the upper surface of the nervous layer of the ordinary type. In the cords they penetrate without dividing the whole of the upper stratum consisting of bipolar neurons and only divide near the border of the lower stratum or in the latter.

I have not been able to find any features in the collar or peduncle cords differing fundamentally from what has been stated here for the cords in general (figs. 19, 20). In the peduncle cord the bipolar cell bodies seem to be particularly common as was stated by BULLOCK. The collar cord appears, diagrammatically, as a rolled-in part of the dorsal nervous layer, where the bottom has the structure of a common cord, the roof that of the epidermal nervous layer in general. I have failed to observe with certainty any unipolar neurons, except the giant cells, cf. below. Structures appearing occasionally which resemble such neurons have always proved to be incompletely stained or obliquely cut sense cells.

It follows from the above that I cannot confirm the previous propositions that the ganglion cells are sharply segregated from the fibre layers and are, more explicitly, assembled outside the nervous layer on its peripheral side, in the collar cord being consequently the nervous component closest to the lumen or lumina. On the contrary, the ganglion cells observed by me are most numerous in the stratum adjacent to the basal membrane, a phenomenon even more obvious in the collar cord than in other parts of the nervous system; further, they are scattered within the fibre layers.

The coelomic nervous system, finally, consists of a one-layered plexus. Its cell bodies are of about the same size as the multipolar neurons described above but of a somewhat more regular circumference (fig. 16). In all cells observed by the author the nucleus is a characteristic narrow oblong shape. As the nuclei of the multipolar and bipolar cells are always spherical, this feature means an interesting morphological difference between cell types which, as we shall see, can be judged for other reasons as belonging to different functional categories. The short fibres described above (p. 156) as extending sideways from the plexus emanate from these cell bodies, one single fibre from each of them. It was not possible to settle definitely if the cells have other processes.

The multipolar neurons forming nets are no doubt association cells for local

VOL. 31 1950

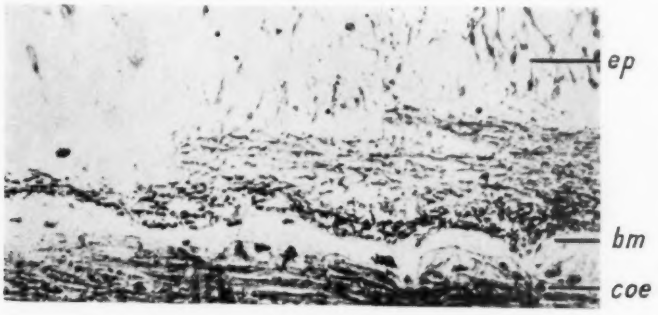

Fig. 17.

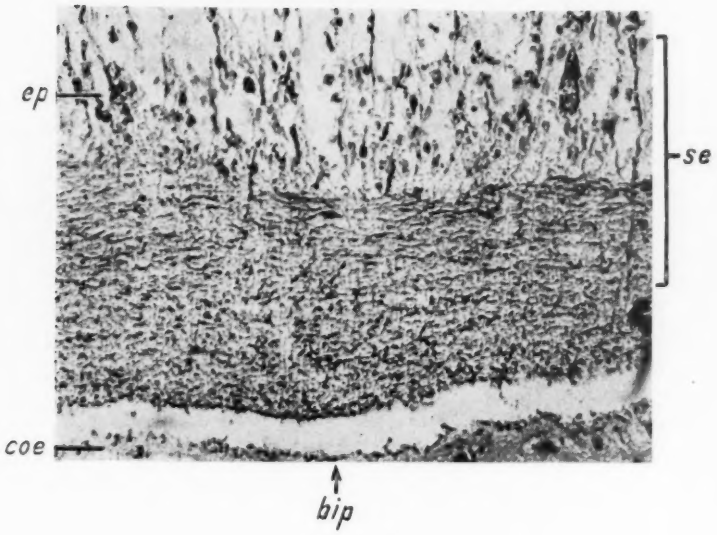

Fig. 18.

Structure of dorsal nerve cord of epidermis in trunk.

Fig. 17. Oblique longitudinal section through margin of cord (right) passing outside the latter through general nerve layer of epidermis (left), showing continuation of general nerve layer as basal stratum of cord and superposition of longitudinal fibres (of bipolar neurons) as upper stratum. 400 ×.

Fig. 18. Median longitudinal section of cord, showing bipolar cell body (in centre of picture), extension of sense cell (near right margin), and longitudinal direction of fibres in large upper part. 350 ×.

For letters, see p. 175.

responses. The occurrence of such neurons in the Enteropneusts has been surmised and hypothetically pictured by VAN DER HORST, BULLOCK, and DAWYDOFF, as large multipolar cells situated on the peripheral side of the nervous layer. These pictures coincide with and have probably been copied from the conditions

ruling in the Echinoderms. The cells now observed are of the same type but much smaller. The fact that within the nerve cords they are placed below a thick layer of bipolar neurons might seem a considerable difference but is probably of no fundamental importance if we bear in mind the above demonstration of the stratification of the cords and the extension of the centrifugal fibres of the sense cells into their basal strata.

The bipolar neurons certainly represent long distance conducting paths.

The branches of the centrifugal fibres of the sense cells can evidently be supposed to conduct impulses to the intermediate neurons.

The position of the neurons of the coelom and the extension of their fibres designate them, in all probability, as motor neurons supplying the body muscles and perhaps the muscles of the ventral vessel as well. It is not clear what is the nature of the fibres piercing the basal membranes and connecting the motor cells with the epidermal and intestinal nervous layers. Are they fibres of the internuncial neurons, or collateral branches of the motor fibres or even special processes of the motor cells? The same questions have been advanced for the corresponding connections theoretically presumed to exist in the Echinoderms between their "hyponeural" and "ectoneural system" (SMITH 1946). Anyway, the latter of these possibilities is probably not true in the present case as motor fibres were often distinctly observed by the writer without any traces of collaterals having been found.

These interpretations coincide with the results obtained by BULLOCK (1940, cf. further the same author 1945) in his experimental studies on the functions of the nervous system in the Enteropneusts.

The problem remains to be discussed: do the motor neurons discerned along the ventral mesentery represent all that exists of a motor system in *Glossobalanus?*

If the motor fibres are, in reality, as short as they appear in the present preparates, it follows that these neurons suffice to innervate restricted parts only of the body musculature, *i.e.* the muscle fibres attached to the ventral mesentery or basal membranes in its vicinity. However, large parts of the body musculature of the trunk and collar, the muscles of the proboscis, of the dorsal vessel, etc., are distant from these structures. Thus it can be concluded that the picture of the motor system presented here is still, in all probability, incomplete.—If the fibres observed by BULLOCK as piercing the epidermal basal membrane in the proboscis, which has been referred to above, really are nerve fibres and represent a diffuse "fibre-by-fibre crossing" of the membrane everywhere, they might be supposed to have some connection with this problem.

In the present slides bodies appear in the peripheral parts of the collar and dorsal trunk cords which resemble the giant cells described by SPENGEL and BULLOCK. However, they are so unclearly stained here that their processes cannot be traced further than through the nervous layer, where they seem to

VOL.
31
1950

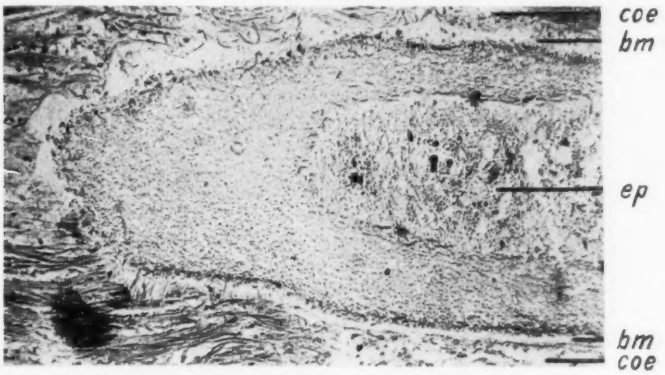

Fig. 19.

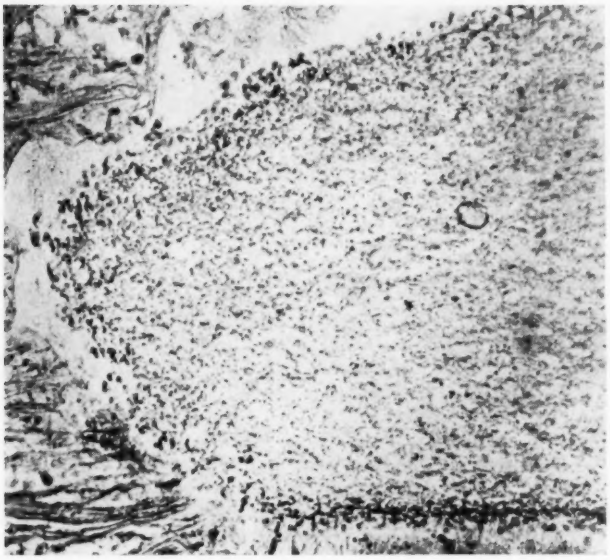

Fig. 20.

Structure of collar nerve cord.

Fig. 19. Oblique longitudinal section of middle part of collar cord, showing peripheral layer with numerous (multipolar) ganglion cells, thick layer of longitudinal fibres (of bipolar cells), and central mass of epithelial cells with cavities. 160 ×.

Fig. 20. Distal part of the same, more magnified. 350 ×.

For letters, see p. 175.

reach at least the vicinity of the basal membrane, and even their nature of neurons could not be ascertained from these preparates. The present author has, consequently, nothing to add to the previous descriptions. These cells have been interpreted by BULLOCK as motor neurons effecting rapid reactions of large parts of the body.

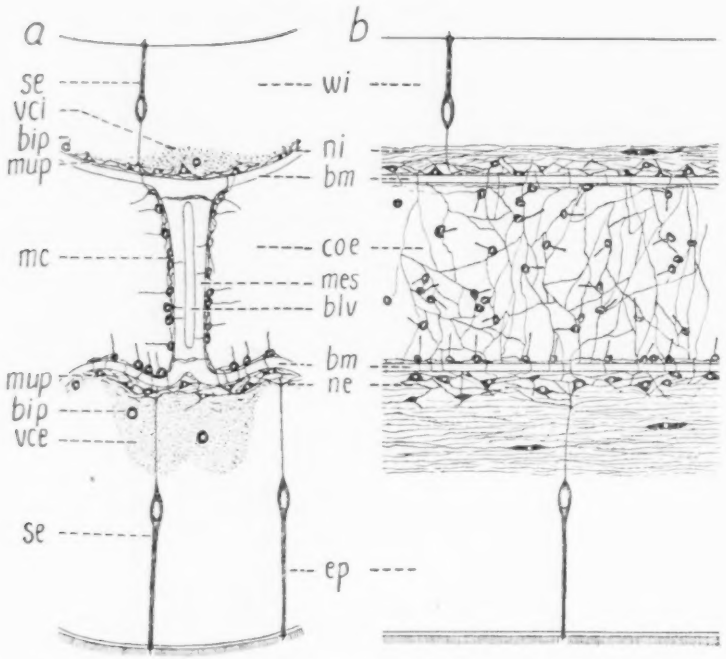

Fig. 21. Diagrams to demonstrate structure of nervous system of Enteropneusts. a: cross section of ventral part of trunk; b: longitudinal section of same part imagined to cut immediately aside ventral mesentery. For letters, see p. 175.

4. SUMMARY AND DISCUSSION.

As this research has been performed almost exclusively on one species, the question appears as to what degree the observations have general validity for the Enteropneusts.

The following features seem so fundamental that deviations of importance are hardly to be expected.

The presence in the epidermis of one type of primary sense cells, their position, structure, and connections; the presence of a simple basally situated plexus composed of multipolar association neurons; the presence of very long bipolar association neurons situated peripherally to the multipolar ones and particularly assembled into stratified dorsal and ventral cords. The probable absence of unipolar neurons (except giant cells) as well as of real nerves or nerve centres.

The presence of a well-developed intestinal nervous layer of the same structure as the epidermal one. The formation of a strong ventral and a feeble dorsal cord, as in the epidermis, is striking.

The presence of a system of motor neurons situated in the coelom along the

VOL. 31 1950

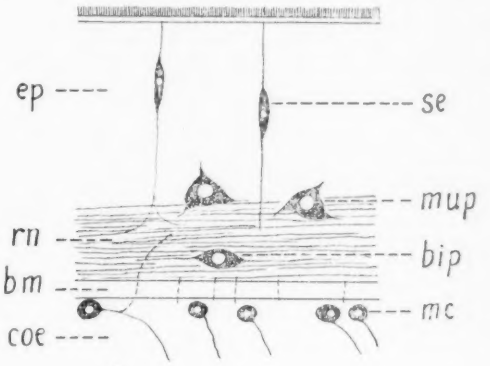

Fig. 22. Diagram of longitudinal section of radial nerve in Asteroidea to demonstrate composition of nervous system in Eleutherozoa. According to MEYER (1906), SMITH (1937, 1946), and CUÉNOT (1948). For letters, see p. 175.

ventral mesentery and connected with the ventral cords of the epidermal and intestinal nervous layers.

The structure of the Enteropneustan nervous system according to the present research is diagrammed in fig. 21.

As is well known, the Enteropneusts occupy an important place in the discussion of the phylogeny of, above all, the Chordates and Echinoderms. (Cf. VAN DER HORST'S admirable survey of 1939.)

Numerous developmental and, in particular, anatomical features have been claimed to connect the Enteropneusts and Chordates. In this discussion the nervous system has played an important part, attempts having been made to homologize the medullary tube of the Chordates with the dorsal (epidermal) nerve cord and, especially, its invaginated collar part in the Enteropneusts.

The arguments for the affinities of the Enteropneusts to the Echinoderms have almost exclusively been based on the striking similitudes of their early development whereas the structure of the adults has been considered too different to permit a comparison with definite results.

The important researches on the Asteroidea of MEYER (1906) and SMITH (1937, 1945, 1946) and the surveys of HANSTRÖM (1928) and CUÉNOT (1948) have been employed, in particular, for the parts of the following account dealing with the nervous system of the Echinoderms. The attempts at staining the nervous system of this group with specific dyes have met with difficulties of a similar type as in the Enteropneusts with the result that some uncertainty remains as to several details. The diagram fig. 22 has been constructed in order to demonstrate its fundamental features.

The general primitivity of the epidermal nervous system of the Enteropneusts corresponds almost exactly to the conditions met with in the Echinoderms. In both groups this system has an epithelial position and is distributed everywhere in the epidermis. Only one type exists of widely distributed primary

sense cells of uncomplicated structure; in some of the more active Echinoderms slightly modified sense cells are aggregated into simple sense organs but largely such organs are absent. Further, the two types of association neurons—bipolar and multipolar ones—of the Enteropneusts agree in position and structure with the corresponding cells of the Echinoderms disregarding minor deviations of probably negligible importance. A degree of simplicity is exhibited by these two nervous systems to which a parallel is not found in other animals with three cell layers but only in the Coelenterates.

The nervous system of the Chordates has generally, and probably rightly, been assumed to have developed from primitive conditions of this type. However, there is no special characteristic motivating a connection with just the Enteropneusts in this respect. It is true that epithelial cells of particularly the collar cord of the Enteropneusts have been compared with the ependym cells of the medullar tube of the Chordates (or some of them even with the glia cells of the latter as they are said to have possibly lost their contact with the basal membrane). However, this seems to be nothing more than another way of expressing the above related facts that the nervous system has an intraepithelial position in the Enteropneusts and that the Chordate one has probably developed from such a primitive state, as no special characteristics in common with these cells have been observed. Of course, the epithelial cells of especially the chords of the Enteropneusts could very well be called ependym cells as, by the way, is often done with the corresponding cells in the Echinoderms. Further, it has been stated as a similarity between the Enteropneusts and Chordates that the ganglion cells of the collar cord of the former group would lie between the fibre layer and the central axis of the cord. This seems dubious already for the reason that the occurrence of such cells here—now disregarding the giant cells—has not been directly observed beyond doubt but is more or less surmised and that the present author has failed to find them in this position but has, on the contrary, found them in the interior and basal parts of the cord.

The only feature in the nervous system of the Enteropneusts that might really be claimed to connect the group with the Chordates is thus the mid-dorsal position of a thickening of the nervous system and the invagination of part of this thickening into a, diagrammatically, tube-like structure. This is, indeed, an interesting phenomenon as it represents almost the only sensible possibility for comparing the dorsal central nervous system of the Chordates with part of the nervous system of another group. However, several other facts have to be taken into consideration in this connection.

In the Enteropneusts there is a mid-ventral cord as well, extending through the entire trunk and here stronger than the dorsal one. The particularly strong invaginated collar cord is formed by the fusion of that ventral cord to the dorsal one. Further, it has been demonstrated here that there are also such

VOL. 31 1950

median cords, the ventral stronger than the dorsal one, in the intestinal layer and that all the cords are formed by an aggregation of long distance conductive fibres. Nobody has succeeded in demonstrating any centres in the cords of the Enteropneusts. On the contrary, the presence of such centres seems improbable to judge by the experiments on the reactions of the Enteropneusts performed by BULLOCK (1940 and 1945).

In the Eleutherozoa the general epidermal nervous layer is thickened into conductive tracts, that is, a "ringnerve" and nerve cords radiating from it (the "ectoneural system"), as is well known. In the Asteroidea they keep an epithelial position but in the Ophiuroidea, Echinoidea, and Holothurioidea the radial cords are situated in the bottom of invaginated tubes, the terminal ends of which are still in connection with the epidermis at least in the Echinoidea. Common to the likewise generally distributed epithelial nervous systems of the Enteropneusts and of, at least, the Eleutherozoa of the Echinoderms is thus a general tendency to form thickened conductive tracts along certain lines and to invaginate parts of these important differentiations; in the latter case the connections with the epidermis remain, often at least, as almost always closed "neuropores" (in some few Enteropneusts the lumen of the tube has been described as continuous).—In the Phylactolaematous Bryozoa and *Asplanchna* (Rotifera) hollow cerebral ganglia occur formed by means of invagination and situated at about the corresponding places as the collar cord of the Enteropneusts (cf. GEWERZHAGEN 1913 and HANSTRÖM 1928). An attempt to interpret these conditions seems premature at present.

The nerve cords of the Enteropneusts and Echinoderms cannot, of course, be directly homologized. Their positions are far too different for that. These respective positions are obviously connected with the different body plans of bilateral animals (Enteropneusts) and radiated ones (Echinoderms) and their different ways of functioning. Thus the superficial resemblance of the medullary tube of the Chordates to the dorsal epidermal nerve cord of the Enteropneusts does not necessarily, in itself, mean much more than that the groups are both of them of a bilateral structure.

The present author feels inclined to agree with the the cautious position taken by BULLOCK (1945, p. 6) when he states that the strength of the argument from the nervous system for the Chordate affinities of the Enteropneusts has been exaggerated but that, on the other hand, nothing in its structure speaks directly against them. Such affinities are certainly to be accepted on the testimony of other anatomical features.

On the other hand, it is evident already from the above account that a comparison between the nervous system of the Enteropneusts and Echinoderms has a far broader and more solid foundation as seen from their fine and gross anatomy. This picture of general correspondence is made even more complete by a comparison of the intestinal nervous systems of the two groups.

The Echinoderms possess a well-developed nervous layer in the intestinal wall continuous with the circumoral ring. Its fine structure is as yet but fragmentarily known. Cigarshaped nuclei considered as belonging to sense cells are abundant in the oesophagus as well as in parts of the stomach and haepatic coeca, in the Asteroidea at least. An intestinal nervous system with primary sense cells has been demonstrated only in the Coelenterates and now in the Enteropneusts among other invertebrate groups. Speculations as to possible connections with the much discussed intestinal nervous tissue in the vertebrates seem altogether premature.

It is true that this similar structure of the nervous systems of the Enteropneusts and Echinoderms does not so far with absolute necessity mean more than a primitive state in common: many general features occur in the Coelenterates as well. However, against this background of general correspondence a comparison of the motor systems of the two groups appears as decisive.

In the mesoderm of the Echinoderms nerve cell layers are situated close below the radial thickenings of the epidermal nervous system, only the basal membrane separating them. This "hyponeural system" has hitherto been considered unique in the animal kingdom. It consists of motor cells certainly rightly considered—though not yet demonstrated—as being connected with the epidermal nervous tissue by fibres piercing the separating membrane. It supplies part of the body musculature. Motor cells have been found in other parts of the mesoderm as well. The correspondence between these characteristic conditions and those just described in the Enteropneusts is striking. We have, in both groups, to deal with motor systems with the unusual situation in the mesoderm and with the same relation to nerve cords of similar structure and origin from the general nervous layers.

Unfortunately, nothing seems to be known with certainty about the ontogenetical development of the nervous tissues in the mesoderm and entoderm of the groups discussed. The terms "mesodermal" and "entodermal nervous system" occasionally used in the literature are thus devoid of a special meaning, only referring to the position of those tissues and saying nothing about a possible similar origin in common.

In the writer's opinion, the demonstrated correspondence between the nervous systems of Enteropneusts and Echinoderms, not only in general but also in positively characteristic features, affords a distinct argument for comparatively close affinities between the two groups.

LITERATURE.

BATESON, W. 1886. Later stages in the development of Balanoglossus Kowalevskii, and morphology of the Enteropneusta. Quart. Journ. Micr. Sci., N. S., 26.

BRAMBELL, R., & COLE, H. A. 1939. The preoral ciliary organ of the Enteropneusta: its

occurrence, structure, and possible phylogenetic significance. Proc. Zool. Soc. London, Ser. B, 109.

BULLOCK, T. H. 1940. The functional organisation of the nervous system of the Enteropneusta. Biol. Bull., 79.

— 1944. The giant nerve fibre system in Balanoglossus. Journ. Comp. Neurol., 80.

— 1945. The anatomical organization of the nervous system of Enteropneusta. Quart. Journ. Micr. Sci., N. S., 6.

CUÉNOT, L. 1948. Anatomie, éthologie et systématique des Échinodermes. Traité de zoologie, publié sous la direction de Pierre-P. Grassé, Tome 11., Paris.

DAWYDOFF, C. 1948. Embranchement des Stomocordés. Ibid.

GEWERZHAGEN, A. 1913. Beiträge zur Kenntnis der Bryozoen. I. Das Nervensystem von Cristatella mucedo Cuv. Zeitschr. Wiss. Zool., 107.

HANSTRÖM, B. 1928. Vergleichende Anatomie des Nervensystems der wirbellosen Tiere. Berlin.

HESS, W. 1937. The nervous system of Dolichoglossus kowalevskyi. Journ. Comp. Neurol., 68.

— 1938. Reactions to light and the photoreceptors of Dolichoglossus kowalevskyi. Journ. Exp. Zool., 79.

HORST, C. J. VAN DER. 1927—1939. Hemichordata. BRONN's Klassen u. Ordnungen d. Tier-Reichs, Bd. 4, Abt. 4, Buch 2, T. 2.

MEYER, R. 1906. Untersuchungen über den feineren Bau des Nervensystems der Asteriden. Zeitschr. Wiss. Zool., 81.

PALMGREN, A. 1948. A rapid method for selective silver staining of nerve fibres and nerve endings in mounted paraffin sections. Acta Zool., 29.

ROMEIS, B. 1948. Mikroskopische Technik. München.

SMITH, J. E. 1937. On the nervous system of the starfish Marthasterias glacialis (L.). Phil. Trans. Roy. Soc. London, Ser. B, 227.

— 1945. The role of the nervous system in some activities of starfishes. Biol. Rev. Cambridge Phil. Soc., 20.

— 1946. The mechanics and innervation of the starfish foot-ampulla system. Phil. Trans. Roy. Soc. London, Ser. B, 232.

SPENGEL, J. W. 1893. Die Enteropneusten des Golfes von Neapel und der angrenzenden Meeres-Abschnitte. Fauna u. Flora d. Golfes v. Neapel, 18, Berlin.

— 1904. Neue Beiträge zur Kenntnis der Enteropneusten. III. Eine neue Enteropneusten-art aus dem Golf von Neapel. Zool. Jahrb., Abt. f. Anat. etc., 20.

ABBREVIATIONS.

bip bipolar ganglion cell.
blv blood vessel.
bm basal membrane.
coe coelom.
dci dorsal nerve cord of intestine.
ep epidermis.
mc motor cell.
mes mesentery.
mup multipolar ganglion cell.
ne nervous layer of ectoderm.
ni nervous layer of intestine.
rn radial nerve.
se sense cell.
vce ventral nerve cord of epidermis.
vci ventral nerve cord of intestine.
wi wall of intestine.

VOL
31
195

ON THE DERMAL CONNECTIVE TISSUE OF THE HEAD OF PETROMYZON

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OL.
31
950

CONTENTS.

1. Development of Larval Aponeurosis 177
2. Metamorphic Changes of the Derma 178
3. Discussion 182
Literature 184
Abbreviations 185

The skin of the Petromyzon larva consists of an epidermis and a dermal aponeurosis. Inside the collagenous bundles of the latter lies a layer of loose connective tissue (STUDNIČKA 1909). The same constitution of the skin is found in the adult, as described by KRAUSE (1923, cf. also RABL 1931, FAHRENHOLZ 1936). During metamorphosis parts of the collagenous layers, forming the larval aponeurosis of the head, change into loose connective tissue, as was shortly mentioned in a previous paper by the present author (JOHNELS 1948). This process is the main subject of this study; a brief description of the formation during ontogeny of the dermal layer of connective tissue will also be given.

1. DEVELOPMENT OF LARVAL APONEUROSIS.

In young embryos (*P. fluviatilis*) there is a thin basal membrane separating the epithelium of the skin from the densely collected cells of the underlying ectomesoderm. Later, when the cells of the ectomesoderm begin to differentiate, mostly forming a loose mesenchymatic tissue, some of the cells remain close to the basal membrane. These cells are evidently fibrocytes, forming new thin layers of collagenous fibres inside the basal membrane. This process can be seen in embryos aged about 500—600 hours.

The dermal layer gradually grows thicker during the larval period. At first

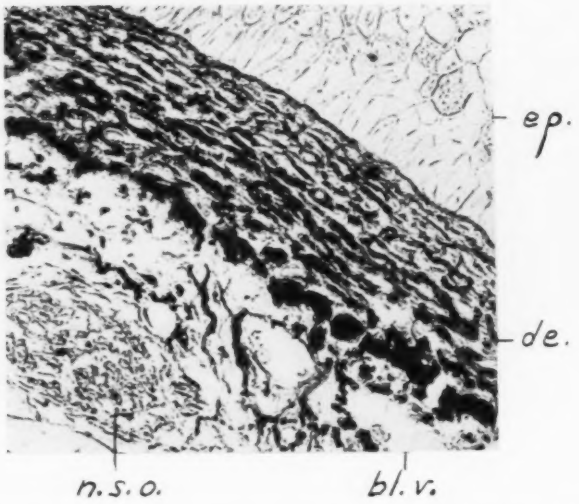

Fig. 1. Section through the skin of Petromyzon rostro-dorsally to the eye. Metamorphic stage 1. 330 ×.

the fibrocytes are not included among the collagenous bundles formed inside the basal membrane. In a larva 13.5 mm. long, however, a few cells are left between the latest formed collagenous bundles. At this stage the thickness of the dermal tissue is about one third of the epithelium. At later stages it grows approximately as thick as one half of the epithelium, at least in the main area of the head. Naturally the epithelium grows thicker at the same time.

The scattered connective tissue cells of Petromyzon which during ontogeny form the cell-free collagenous layers underneath the basal membrane are not as regularly arranged as in Myxine embryos (cf. HOLMGREN 1946). The dermal parts of the skin of Myxine and the larval Petromyzon are, however, built up mainly in the same way. The surface layer bordering on the epithelium consists of thin bundles of collagenous fibres. No cells are merged in between these fibres. Inside this layer lies the thicker part of the dermal tissue. This part consists of strong collagenous bundles, between which there are scattered connective tissue cells, presumably fibrocytes.

2. METAMORPHIC CHANGES OF THE DERMA.

Very early during the metamorphosis of Petromyzon (planeri) there are changes in parts of the dermal tissue of the head. Around the nasal aperture and inside the buccal epithelium of the "upper lip" the collagenous bundles of the subepithelial derma begin to separate from each other (cf. fig. 1). The

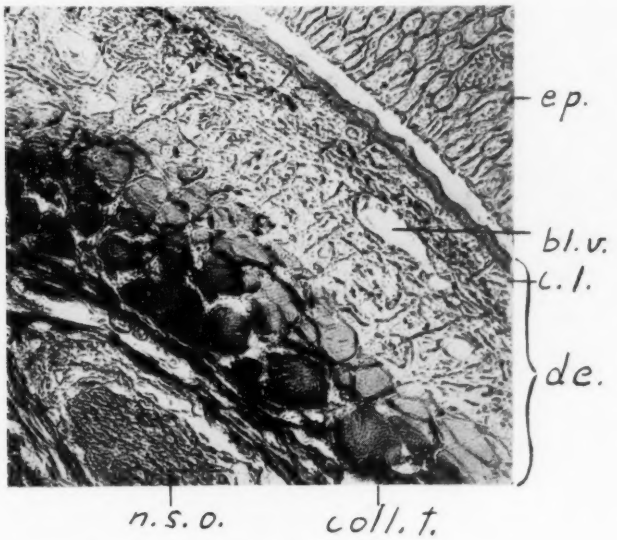

Fig. 2. See fig. 1. Metamorphic stage 2.

fibrocytes become easily visible in the spaces between the bundles. This is the first sign of the loosening of the dermal collagenous tissue to take place in the rostral part of the head. The cells which are freed in the derma inside the buccal epithelium later proliferate very abundantly and participate in the formation of some of the rostral cartilage elements (cf. JOHNELS 1948).

At a somewhat later stage the loosening of the derma is spread over a greater area, finally affecting the rostral part of the head entirely and the groove where the gill slits open. It is at first especially pronounced on the surface covering the eye where the cornea is finally formed (cf. KEIBEL 1927—28). Parts of the larval derma in the branchial groove participate in the formation of cartilage elements (cf. JOHNELS 1948).

Inside the layer of loosening dermal tissue there is a remarkable increase in the number of blood vessels (fig. 1 *bl. v.*). The spaces between the bundles of collagenous fibres gradually grow wider; at the same time the number of nuclei in the spaces increases as a result of numerous cell divisions. During the first phases of this process the bundles are still continuous, but later they break into pieces. At this stage blood vessels also occur within the area of the original dermal tissue (fig. 2 *bl. v.*). Immediately beneath the epithelium a very thin layer of collagenous tissue persists. Inside this stratum free connective tissue cells gather, forming an epithelium-like layer (fig. 2 *c. l.*). The collagenous bundles situated immediately underneath this layer disappear completely. At the same time the cells grow sparser in this place as they gather

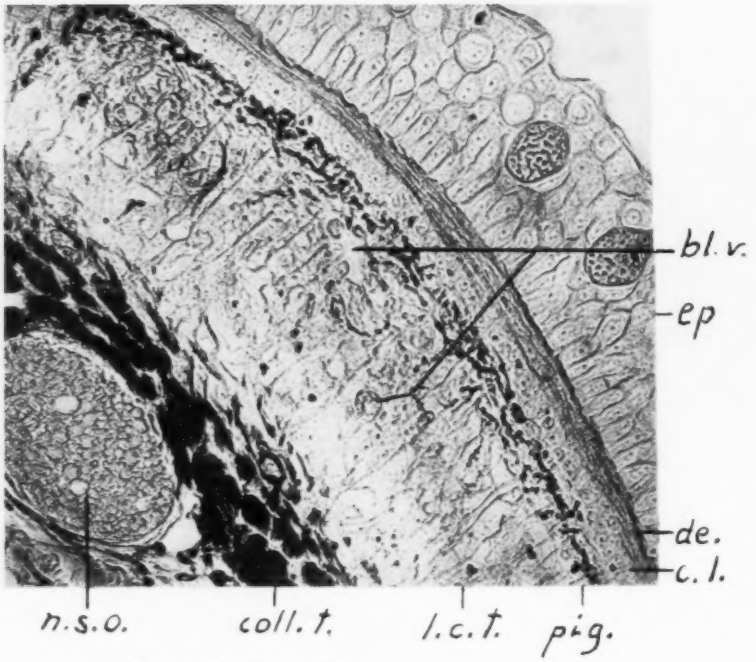

Fig. 3. See fig. 1. Metamorphic stage 3.

towards the superficial stratum. The remaining cells are of an embryonic type, connected with each other by rather long and thin cell processes. In the larval derma as well as in the adult there are thin connective tissue fibres running perpendicularly towards the dermal surface. These fibres are still visible at quite late stages of the dissolving process (figs. 2 and 3). In the proximal part of the original derma the collagenous bundles do not break down completely (fig. 2 *coll. t.*). They persist as fragments surrounded by scattered connective tissue cells. The innermost layer of the original aponeurosis merges with the more or less regular bundles of collagenous fibres of the subdermal tissue.

At the late metamorphic stage now described the dissolution of the tissue ceases and a restoration of the derma begins. The cells, densely crowded beneath the persisting subepithelial stratum, form new layers of collagenous bundles (fig. 3 *de.*). The sparse cells left in the original middle portion of the aponeurosis form a loose connective tissue (figs. 3, 4 *l. c. t.*). In the inner part of this layer fat cells are present, but not as abundantly as is the case in the subdermal layer in, for instance, the branchial region. In the adult the blood vessels of this layer seem to be less frequent than in metamorphic stages. The persisting fragments of collagenous bundles inside the layer of loose connective tissue are restored and form a rather thick and relatively distinct stratum

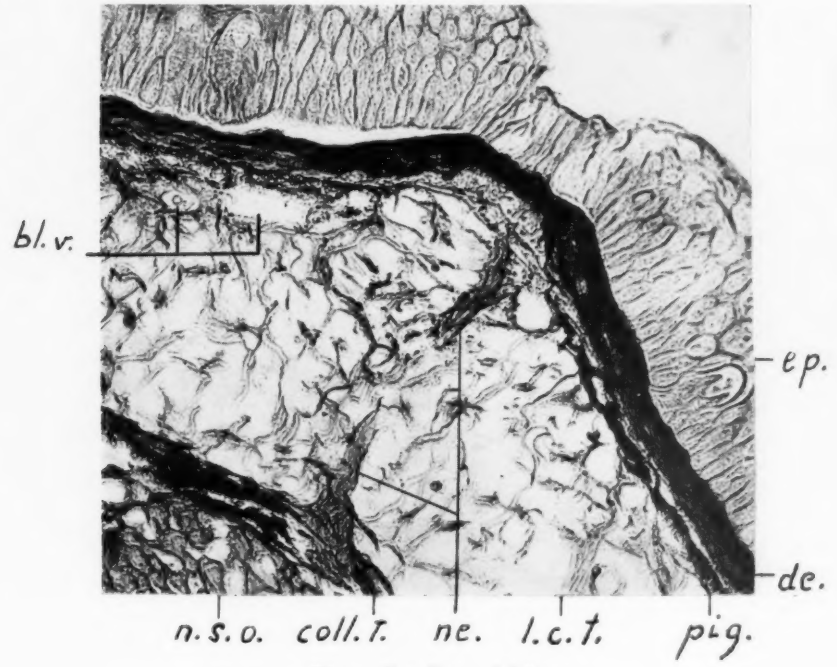

Fig. 4. See fig 1. Adult.

together with new-formed bundles and bundles of the subdermal tissue (fig. 4 *coll. t.*). This last mentioned stratum borders on the sparse connective tissue which is left in the underlying parts of the head after metamorphosis (cf. JOHNELS 1948).

During the dissolving phase the sensible nerve branches, running from the subdermal nerve plexus through the derma towards the endings in the epithelium (cf. RAZZAUTI 1916), are prolonged. The subdermal nerve plexus remains inside the tissue of the original derma (fig. 5).

In the larva there is a layer of pigment cells inside the dermal tissue. This pigment layer is thick on the dorsal and lateral sides of the animal but thin on the ventral. During the dissolving phase the area of the aponeurosis, as seen in a transverse section, grows thicker than in the original larval stage (figs. 1—4). At first the pigment layer persists on the inner side, but soon most of the pigment cells start migrating towards the superficial layers (fig. 3 *pig.*). Finally most of them are gathered underneath the dense fibrous layer, constituting the derma of the adult (fig. 4 *pig.*). A few pigment cells are scattered in the loose connective tissue underneath this layer, and a rather incomplete layer of pigment cells is present inside the basal collagenous membrane.

The process of dissolution of the larval derma occurs within a limited area

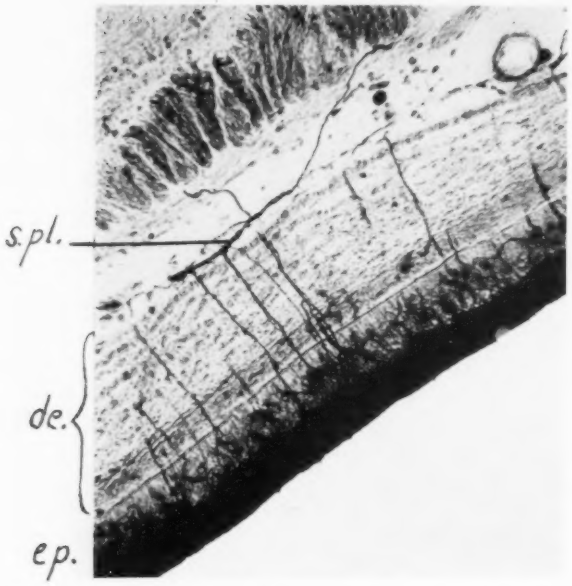

Fig. 5. Section through the skin of Petromyzon ventrally to the eye. Metamorphic stage. Golgi. 125 ×.

of the head. This area includes the rostral part. Dorsally and ventrally around the median line the area protrudes caudad for a distance. Laterally the rostral area continues caudad in a long process, including the branchial groove of the larva (fig. 6). The formation of three different layers does not occur in the branchial groove, where a single aponeurosis is built up during the late metamorphic stages. The formation of three different layers is limited to the rostral parts of the head, in front of the branchial groove. Caudally to the approximate bordering line of this three-layer formation the adipose tissue inside the derma is the subdermal tissue of the larva.

3. DISCUSSION.

During metamorphosis the shape of the rostral part of the head is especially changed. The "upper" and "lower lips" of Ammocoetes are prolonged rostrad and transformed into the sucking mouth of the adult, and the eyes grow large. In the branchial region the gill groove of the larva is flattened out. These processes of growth may be a sufficient explanation of the fact that the dissolution of the dermal tissue occurs within these areas. The original dermal tissue might be too resistant to allow great changes of shape.

VOL. 31 1950

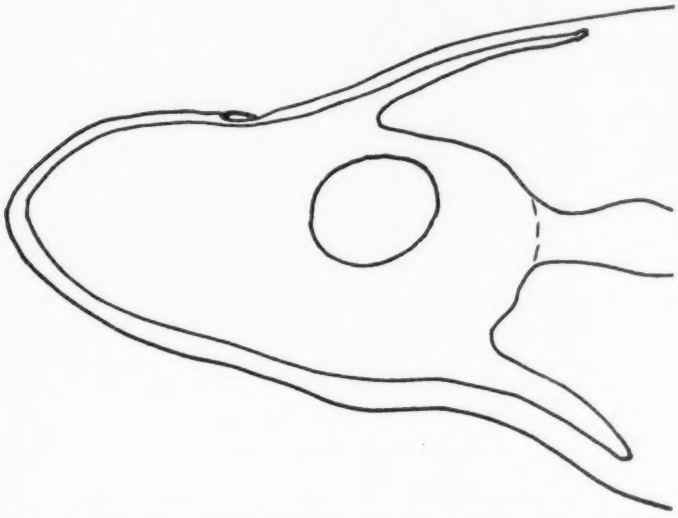

Fig. 6. Petromyzon, anterior part of the head. See text p. 182. 10 ×.

But the restitution of the tissue during the late phases of metamorphosis does not everywhere result in a single aponeurosis. Instead three different layers are formed within a limited area of the head. These three layers as a whole are homologous with the larval derma. The area of the three-layer formation approximately coincides with the outline of the endoskeletal parts of Ammocoetes, compared by GASKELL (1900), STENSIÖ (1927), HOLMGREN and STENSIÖ (1936) and JOHNELS (1948) with the cephalaspid endocranium. This coincidence suggests a connection between the process of growth during metamorphosis of the derma of the head and the underlying endoskeletal parts.

In the opinion of the present author there are reasons to believe that the ancestors of Petromyzon possessed a more developed endoskeleton than the species now living (STENSIÖ 1927, 1932, HOLMGREN and STENSIÖ 1936, JOHNELS 1948).

In Cephalaspids there was an exoskeleton covering the rostral part of the body and the head. This exoskeleton consisted of three different layers, a superficial, a middle and a basal layer (STENSIÖ 1927, 1932, DENISON 1947). According to STENSIÖ the exoskeleton had arisen in the derma and occupied this part of the skin in its entire thickness. STENSIÖ is, however, of the opinion that the most basal part of the basal layer included laminae formed by the underlying endoskeleton. In the head of the adult Petromyzon there are three layers formed in the original derma of the larva, as described above. The superficial layer of Cephalaspids may correspond to the dermal aponeurosis of the

adult Petromyzon, the middle layer of the Cephalaspid exoskeleton to the layer of loose and adipose tissue of Petromyzon, and the basal layer of the Cephalaspid exoskeleton to the innermost layer of collagenous tissue of Petromyzon. As in Cephalaspids the basal layer of Petromyzon is fused with parts of the underlying tissue. In Cephalaspids the middle layer was strongly vascularised, and the vessels conducting blood to and from this vascular plexus passed through the basal layer. The middle layer of Petromyzon also contains blood vessels, communicating with the vascular system of the body through the inner layer of collagenous tissue.

Quite recently WESTOLL (1945) has pointed out the possibility that the Cephalaspids went through a metamorphosis, an opinion which was expressed to the present author several years ago by Professor E. STENSIÖ. Of the nature of this assumed metamorphosis very little can be known. Additional facts supporting this theory have been produced by DENISON (1947). WESTOLL (1945, p. 345) writes: "It may therefore be deduced that the ossification of the exoskeleton of cephalaspids probably took place only at full growth; — — —." However he mentions that the superficial layer of the exoskeleton may have begun the ossification earlier.

The relationship between Petromyzon and Ostracoderms seems to be well documented (STENSIÖ 1927, 1932, HOLMGREN and STENSIÖ 1936). Provided that the ancestors of Petromyzon—like Cephalaspids—were once in the possession of a real exoskeleton that was formed during a metamorphosis, it seems possible that the separation of three different layers of the larval derma of Petromyzon during metamorphosis, peculiar to the rostral part of the head, may be a remnant of such an exoskeleton. The reduction of the endoskeleton of Petromyzon during phylogeny was in that case accompanied by a reduction of the exoskeleton.

VOL. 31 1950

LITERATURE.

DENISON, R. H. 1947. The exoskeleton of Trematapsis. Amer. J. Sc. 245.

FAHRENHOLZ, C. 1936. Die sensiblen Einrichtungen der Neunaugenhaut. Zschr. mikrosk.-anat. Forsch. 40.

GASKELL, W. H. 1900. On the origin of Vertebrates, deduced from the study of Ammocoetes, VIII. J. Anat. Physiol. 34.

HOLMGREN, N. 1946. On two embryos of Myxine glutinosa. Acta Zool. 26.

HOLMGREN, N. und STENSIÖ, E. 1936. Kranium und Visceralskelett der Akranier, Cyclostomen und Fische. Bolk, Göppert, Kallius, Lubosch, Handb. d. vergleichenden Anatomie. IV. Berlin u. Wien.

JOHNELS, A. G. 1948. On the development and morphology of the skeleton of the head of Petromyzon. Acta Zool. 29.

KEIBEL, F. 1927—28. Beiträge zur Anatomie, zur Entwicklungsgeschichte und zur Stammesgeschichte der Sehorgane der Cyclostomen. Zschr. mikrosk.-anat. Forsch. 12.

KRAUSE, R. 1923. Mikroskopische Anatomie der Wirbeltiere in Einzeldarstellungen. 4. Berlin u. Leipzig.

RABL, H. 1931. Integument der Anamnier. Bolk, Göppert, Kallius, Lubosch, Handb. d. vergleichenden Anatomie. I. Berlin u. Wien.

RAZZAUTI, A. 1916. Struttura del tegumento dei Petromizonti. Nervi ed organi cutanei di senso. Arch. Italiano Anat. Embriol. 15.

STENSIÖ, E. 1927. The Downtonian and Devonian vertebrates of Spitzbergen. I. Skrifter om Svalbard og Nordishavet. 12.

— 1932. The cephalaspids of Great Britain. London.

STUDNIČKA, F. K. 1909. Vergleichende Untersuchungen über die Epidermis der Vertebraten. Anat. H. 39.

WESTOLL, T. S. 1945. A new cephalaspid fish from the Downtonian of Scotland, with notes on the structure and classification of ostracoderms. Trans. Roy. Soc. Edinburgh. 61, II. 1948.

ABBREVIATIONS.

bl. v. blood vessel.
c. l. cellular layer.
coll. t. collagenous tissue.
de. dermal tissue.
ep. epithel.
l. c. t. loose connective tissue.
nc. lateralis nerve.
n. s. o. nervus supraorbitalis.
pig. pigment layer.
s. pl. subdermal nerve plexus.

VOL.
31
1950

ÜBER DAS WACHSTUM DER KOPFORGANE UND DIE ENTSTEHUNG VON MIKROPHTHALMIE BEI ISOLIERTEN KOPFSTÜCKEN UND ZIRKULATIONSLOSEN KEIMEN VON TRITON ALPESTRIS

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INHALTSÜBERSICHT.

I. Einleitung und Problemstellung 188
II. Material und Methode 190
1) Material 190
2) Ausführung der Operationen 190
3) Weiterzucht und Kontrolle 192
4) Messmethode 193
a) Durchmesser der Linse 193
b) Querschnitt durch die Retina 193
c) Querschnitt durch das Vorderhirn 193
d) Querschnitt durch das Zwischenhirn 194
e) Das Volumen des Riechepithels 194
f) Das Volumen des Ohres 194
III. Das normale Wachstum der Kopforgane 194
1) Wachstum und Wachstumskurven 195
2) Die Wachstumsgeschwindigkeit 198
IV. Experimente 200
1) Isolierte Kopfstücke 200
2) Exstirpation des Herzblastems 201
3) Vergleich der Ergebnisse 203
a) Augen 203
b) Andere Kopforgane 208
c) Vergleich der Wachstumskurven 211
d) Zirkulationsausfall als Ursache der Wachstumshemmungen 213
4) „Halbparabiosen" 214
5) Der Einfluss des Blutes als Dauerwirkung 218
6) Ursachen der Mikrophthalmie 220
a) Verminderung der Atmung 220
b) Herabsetzung der Ernährung 222
V. Diskussion 224
1) Sauerstoffzufuhr 227
2) Nutritive Wirkung 227
3) Hormonale Wirkung oder Ernährung 228
VI. Zusammenfassung 228
VII. Literaturverzeichnis 229

Die Anregung zu dieser Arbeit verdanke ich meinem verehrten Lehrer Herrn Prof. Dr. E. HADORN. Ihm möchte ich für die zahlreichen Anregungen und Ratschläge sowie für das Interesse, mit dem er meine Arbeit stets verfolgte, herzlich danken.

I. EINLEITUNG UND PROBLEMSTELLUNG.

Augenmissbildungen wie Anophthalmie, Mikrophthalmie und Cyclopie treten als spontane Fehlleistungen in der Wirbeltierentwicklung relativ häufig auf. Viele dieser Anomalien lassen sich auf Gene zurückführen, vor allem auf Letal- und Semiletalfaktoren, die neben anderen Phänen auch abnormale Augenentwicklung bewirken. Die phaenotypische Auswirkung solcher Gene kann zur vollkommenen Anophthalmie, aber auch nur zu einer leichten Verkleinerung der Augen führen, wobei alle Zwischenstufen vorkommen (Mikrophthalmie), oder sie kann sich auf einen Teil des Auges beschränken (Cataract, stäbchenlose Retina u. a.). Solche Phänomene wurden vorwiegend bei Haus- und Laboratoriumstieren, aber auch beim Menschen von verschiedenen Autoren beschrieben, so für die Maus (CHASE and CHASE 1941, P. HERTWIG 1942), für die Ratte (H. D. KING 1931, HAIN 1933), für das Meerschweinchen (PICTET et FERRERO 1940, O. N. EATON 1937), für das Schwein (ROBERTS 1948), für das Haushuhn (LANDAUER 1933, JEFFREY 1941), für die Taube (HOLLAENDER 1948) und für den Menschen (HUMPHREY 1924, FRANCESCHETTI u. SEEFELDER 1930, L. H. CARRIS 1932, ASHLEY 1947).

Bei verschiedenen Tieren gelang es, durch geeignete Behandlung der Eltern bei den Nachkommen ähnliche Missbildungen zu erzeugen. So trat Mikrophthalmie bei den Nachkommen von Mäusen auf, die vor der Kopulation mit Röntgenstrahlen behandelt worden waren (BAGG and LITTLE 1924). HALE (1933) beschreibt einen Wurf anophthalmer Ferkel, deren Mutter mit einer Diät gefüttert wurde, der das Vitamin A fehlte. HALE erachtet aber diese Erscheinung nicht als erblich. GUYER und SMITH (1918) injizierten in trächtige Kaninchen Hühnerblut, das auf Linsenextrakt im Sinne einer Sensibilisierung behandelt worden war. Viele dieser Jungen wiesen abnormale Augen und Linsen auf.

Endlich ist es möglich, durch Behandlung von Tieren während der Embryonalentwicklung verschiedene Augenmissbildungen zu erzeugen. ADELMANN (1930) entfernte einen Teil der vorderen Medullarplatte bei *Amblystoma* und bewirkte so Mikrophthalmie. In ähnlicher Weise entstand bei STOCKARDS (1913) Versuchen über die Lokalisation der Augenanlage in vielen Fällen Mikrophthalmie, da ein Teil der praesumptiven Augenanlage entfernt wurde. Von besonderer Bedeutung waren STOCKARDS (1907, 1909) Versuche mit chemischen Mitteln an *Fundulus*, wobei Cyclopie auftrat. LOEB (1915) erzeugte durch Kreuzung der Fische *Fundulus* und *Menidia* mikrophthalme Gattungsbastarde. BARDEEN (1907) bestrahlte Krötenspermien mit Röntgenstrahlen. Die Larven, die sich aus den damit befruchteten Eiern entwickelten, wiesen neben anderen Anomalien auch Augenmissbildungen auf. TRAMPUSCH (1946) bestrahlte Axolotl-Embryonen im Gastrula-Stadium mit Röntgenstrahlen, wor-

VOL. 31 1950

auf die Larven in der Folge mikrophthalm oder anophthalm wurden. FISCHEL (1921) beschreibt sodann eine Anzahl spontaner Augenmissbildungen bei *Salamandra maculosa* und erklärt ihre Ursachen auf entwicklungsphysiologischer Grundlage.

Aus diesen Fällen erblicher und umweltbedingter, spontaner und künstlich hervorgerufener Mikrophthalmie geht hervor, dass die Entstehung dieser Anomalie auf zwei Wegen erfolgen kann:

1) Es gibt eine „*frühbedingte Mikrophthalmie*". Dabei hat man der Tatsache Rechnung zu tragen, dass normale Augen nur dann entstehen, wenn während der Gastrulation und Neurulation ein normaler Kopforganisator seine induzierende Wirkung ausübt. Experimentelle Eingriffe zeigen, dass Schädigungen am Kopfdarmdach zu Fehlleistungen von Hirn- und Augenanlagen führen. Einzelne Typen erblicher Mikrophthalmie können in dieser Art als sekundäre Phaene eines Organisatorschadens gedeutet werden. Charakteristisch für diese Art von Mikrophthalmie ist die Tatsache, dass sie meist zusammen mit Cyclopie und Synophthalmie vorkommt (HADORN 1945).

2) Die abnormale Entwicklung der Augen kann aber auch erst später einsetzen („*Spätbedingte Mikrophthalmie*"). Die Augenblasen werden in diesem Fall zuerst normal ausgebildet; dann kommt es zu einem Wachstumsstillstand und in der Folge können die Augen ganz oder teilweise degenerieren. Es versteht sich von selbst, dass in diesem Fall neben Mikrophthalmie weder Cyclopie noch Synophthalmie auftreten. In diesem Zusammenhang müssen die Beobachtungen von LOEB (1915) an mikrophthalmen Gattungsbastarden von *Fundulus* und *Menidia* erwähnt werden, wo sich die Augen zuerst normal entwickeln und später sekundär degenerieren. H. E. CHASE und E. B. CHASE (1941) beschreiben einen Letalfaktor bei Mäusen, der Anophthalmie bedingt. Auch in diesem Fall wird eine Augenblase gebildet; aber später wird ihre Entwicklung gehemmt. Dadurch bleibt sie zu weit von der Epidermis entfernt, sodass keine Linse induziert werden kann. EIGENMANN (1909) untersuchte die Augenentwicklung bei blinden Höhlentieren. Bei diesen werden die Augen zuerst angelegt, degenerieren aber später teilweise oder vollständig. Solche Fälle sind für alle Wirbeltierklassen mit Ausnahme der Vögel bekannt.

HADORN (1945) hat gefunden, dass Kopfstücke von *Triton taeniatus* und *T. alpestris*, die im Schwanzknospenstadium isoliert wurden, in späteren Stadien mikrophthalm werden. Während die früher genannten Experimente und Eingriffe an Larven in frühembryonalen Stadien (Gastrula- und Neurulastadium) ausgeführt wurden, also nur über die „frühbedingte Mikrophthalmie" Aufschluss geben können, war es nach dieser Beobachtung möglich, eine „spätbedingte Mikrophthalmie" entwicklungsphysiologisch zu untersuchen.

In der vorliegenden Arbeit wurden die Experimente HADORNS (1945) in dem Sinne erweitert, dass die Keime an verschiedenen Stellen quer durchschnitten wurden (Schnitt auf Höhe des Ohrbläschens, zwischen Kiemen- und

Vornierenwulst und drei Somiten weiter hinten. (Abb. 7) und dass verschiedene Stadien für diese Operation verwendet wurden.

In diesem Zusammenhang sind folgende Fragen von besonderem Interesse:

1) Lässt sich diese Art von Mikrophthalmie doch noch auf einen Organisatordefekt zurückführen?

2) Welches sind die Unterschiede qualitativer und quantitativer Natur zwischen diesen Augen und den normal entwickelten Augen?

3) In welchem Entwicklungsstadium beginnt die Anomalie aufzutreten?

4) Wie verhält sich das relative Wachstum der Kopforgane (Auge, Riechepithel, Gehörorgan) und der Kopfbreite zum ganzen Körper. Diese Frage interessierte uns bei der Entwicklung der normalen Tiere wie auch für die Kopfstücke und zirkulationslosen Keime.

5) Wie verändert sich das Verhältnis der Augenteile (Linse und Retina) im Laufe der Entwicklung, sowohl bei den normalen Keimen und Larven, wie bei Kopfstücken und zirkulationslosen Keimen.

6) Welches sind die mittelbaren Ursachen dieser Erscheinungen?

VOL. 31 1950

II. MATERIAL UND METHODE.

1) Material.

Die Versuche wurden mit *Triton alpestris* durchgeführt. Die Tiere stammten zum Teil aus den Lehmgruben am Fusse des Ütliberges, zum Teil aus der Gegend des Katzensees bei Zürich. Sie wurden während der Monate April bis Juni in grösseren Becken und Aquarien gehalten und mit Milz gefüttert. Die Eiablage war während der ganzen Zeit regelmässig. Die Qualität der Eier war bis Anfang Juni gut.

2) Ausführung der Operationen.

Embryonen von *Triton alpestris*, die das Glaesner-Stadium 22/23, resp. 24 (Abb. 1) erreicht hatten, wurden in sterilem Brunnenwasser enthüllt. Darauf wurden zwei Keime von möglichst gleichem Aussehen in eine Schale mit Agarboden in sterile Holtfreter-Lösung übertragen. In diesem Gefäss wurde der eine der beiden Keime quer zerschnitten (S. 200), resp. sein Herzblastem exstirpiert (S. 201), der andere als Kontrolle weitergezüchtet. Dadurch wurde erreicht, dass möglichst gleichartige Einflüsse auf das Kopfstück resp. das cardiectomierte Tier wie auf die Kontrolle wirkten (Temperatur, chemische Einflüsse etc.).

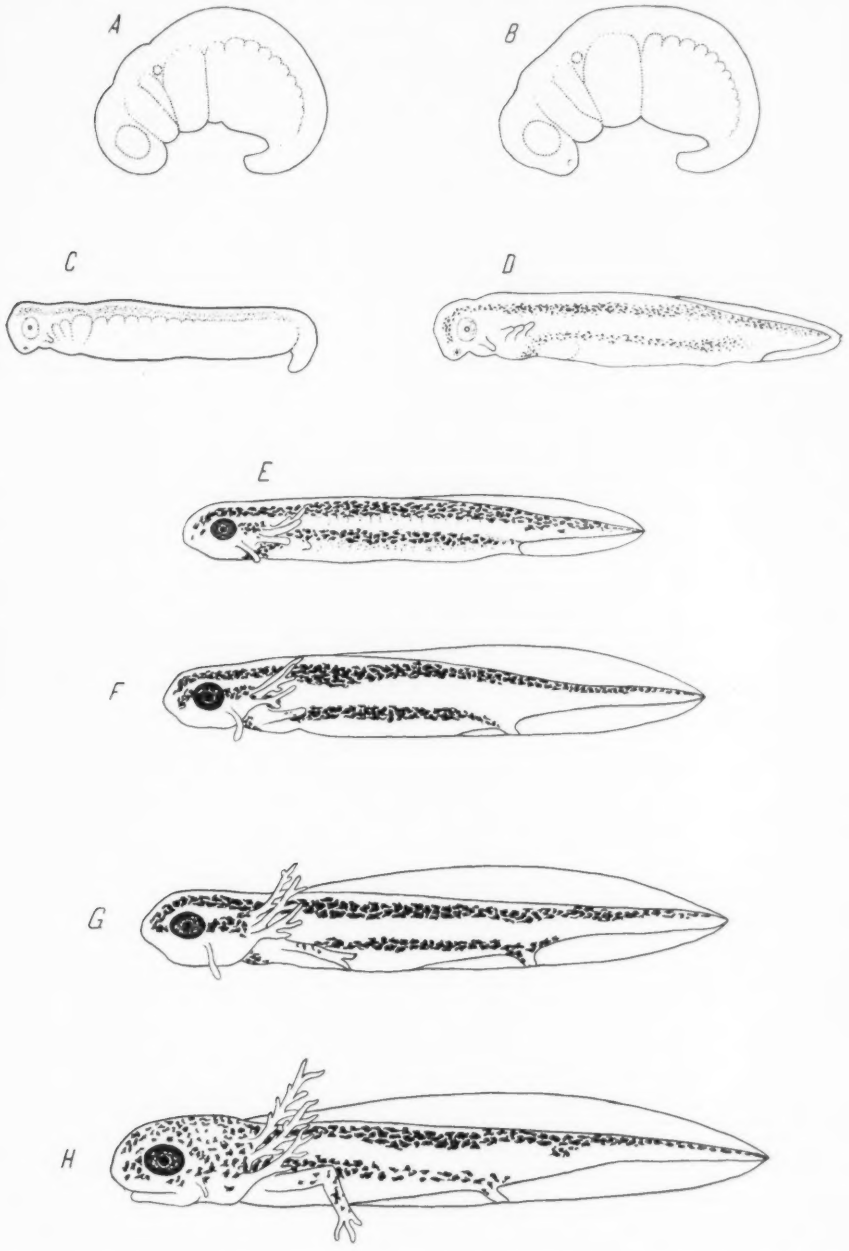


Abb. 1. Operations- und Mess-Stadien. (Nach L. GLAESNER.) A: Stadium 23, wurde verwendet für die Herstellung von isolierten Kopfstücken. B: Stadium 24, wurde gewählt für die Herzexstirpationen. C: Stadium 27. D: Stadium 30. E: Stadium 33. F: Stadium 37. G: Stadium 39. H: Stadium 42.

3) Weiterzucht und Kontrolle.

Beide Tiere, sowohl ein Kopfstück, resp. ein cardiectomierter Keim, wie das Kontrolltier, wurden durchschnittlich alle zwei Tage kontrolliert und gemessen. Für die Datierung der Messung war das Stadium der Kontrolle massgebend, d. h. wenn diese ein bestimmtes Stadium erreicht hatte, wurden beide Tiere in einer gleichen Schale gemessen.

Die Messungen wurden ausgeführt, wenn das Kontrolltier folgende in Abb. 1 dargestellte Glaesner-Stadien erreicht hatte (GLAESNER 1925):

1) Stadium 27: Erscheinen des Pigmentes an Kopf und Rücken.
2) Stadium 30: Kiemen stummelförmig, Herzpulsationen.
3) Stadium 33: Vorderextremität als Höcker entwickelt. Blutzirkulation im Kopf feststellbar.
4) Stadium 37: Die Vorderextremität ist stummelförmig.
5) Stadium 39: Zwei Zehen der Vorderextremität ausgebildet.
6) Stadium 42: Drei Zehen der Vorderextremität ausgebildet. Die Tiere stehen kurz vor der ersten Nahrungsaufnahme. Der Darm ist gut sichtbar. Der Balancer ist zurückgebildet.

VOL. 31 1950

Während der ganzen Beobachtungszeit wurden die Tiere im Thermostaten bei einer Temperatur von 18° ± 1° gehalten. Wie nach KNIGHT (1938) zu erwarten war, zeigte die Kontrolle der Entwicklung, dass die Entwicklungsgeschwindigkeiten bis auf wenige Ausnahmen bei allen Tieren gleich waren.

Die Intervalle zwischen zwei Messungen waren die folgenden:

Vom Gl.-Stad. 22|23 zum Gl.-Stad. 27 ..... 2 Tage,
vom Gl.-Stad. 27 zum Gl.-Stad. 30 ..... 2 Tage,
vom Gl.-Stad. 30 zum Gl.-Stad. 33 ..... 2 Tage,
vom Gl.-Stad. 33 zum Gl.-Stad. 37 ..... 2 Tage,
vom Gl.-Stad. 37 zum Gl.-Stad. 39 ..... 2 Tage,
vom Gl.-Stad. 39 zum Gl.-Stad. 42 ..... 6 Tage.

Die ganze Beobachtungszeit betrug also 16 Tage und beschränkte sich auf die Entwicklungszeit vor der Nahrungsaufnahme. In diesem Zeitpunkt mussten die Beobachtungen abgebrochen werden, weil die operierten Tiere der meisten Versuchsserien nicht fressfähig waren und damit die physiologischen Bedingungen in den Kopfstücken, resp. zirkulationslosen Larven, und in den Kontrollen ungleich geworden wären.

Die Wunden an den zerschnittenen Keimen verheilten in ein bis zwei Tagen. Während dieser Zeit war die Gefahr von Infektionen recht gross. Trotz sauberer Arbeit starben anfangs ein drittel bis die Hälfte der Kopfstücke an Infektionen. Im zweiten Versuchsjahr gelang es, durch Verwendung von „Elkosin" die Infektionsrate herabzusetzen.

4) Messmethode

Die Messungen von Augendurchmesser und Kopfbreite wurden an lebenden Tieren durchgeführt. Mit Hilfe des Zeichenapparates wurden die operierten Tiere und die Kontrollen von der Seite und von oben bei 48-facher Vergrösserung gezeichnet. Nachher wurde an Hand von Skizzen der grösste Durchmesser des ellipsoidischen Auges und die Linsendistanz (= Kopfbreite) bestimmt.

Die Entwicklung der inneren Organe, wie Gehirn, Riechepithel, Gehörorgan, sowie der Augenteile, wie Retina und Linse, wurde an Hand von mikroskopischen Schnitten verfolgt. In allen oben genannten Stadien wurden einige der operierten Tiere mit ihren Kontrollen in Bouin'schem Gemisch fixiert. Im Endstadium der Beobachtung (Gl.-Stad. 42) wurden am meisten, d. h. für jede Versuchsserie mindestens 15 Tiere fixiert. Um die Messungen an den Schnitten quantitativ zu erfassen und miteinander zu vergleichen, war es nötig, darauf zu achten, dass die Schrumpfung infolge der Fixierung bei allen Objekten möglichst gleich war. Sowohl beim Fixieren wie beim Hinaufführen über Alkohol, Methyl-Benzoat-Celloidin in Paraffin wurde dafür gesorgt, dass die Objekte während gleich langer Zeit in den verschiedenen Flüssigkeiten und Stufen gehalten wurden.

Die Untersuchung der mit Haemalaun-Eosin gefärbten Schnitte erstreckte sich auf folgende Organe:

a) *Durchmesser der Linse.*

Die Schnitte, auf denen die Linse festzustellen war, wurden gezählt. Wegen der kugeligen Form der Linse ist der mittlere dieser Schnitte ihr grösster Querschnitt. Dieser Transversalschnitt durch das Auge wurde mit dem Zeichenapparat bei 220-facher Vergrösserung gezeichnet und auf der Skizze der Linsendurchmesser gemessen.

b) *Querschnitt durch die Retina.*

Im gleichen Schnitt wie bei (a) wurde auch der Querschnitt durch die Retina gemessen. Seine Umrisse wurden mit dem Zeichenapparat gezeichnet und der Flächeninhalt mit dem Planimeter bestimmt. Um das Ausmass der Wachstumshemmung der verschiedenen Organe vergleichen zu können, war es nötig, die Messungen auf ein lineares Mass zu reduzieren.

c) *Querschnitt durch das Vorderhirn.*

Für diese Messung wurde derjenige Schnitt herausgesucht, in dem sich die Vereinigung der beiden Seitenventrikel feststellen lässt. Dieser Querschnitt wurde gezeichnet und nachher die Schnittfläche des Vorderhirns ohne Ven-

trikel planimetriert. Es wurde also nur der Querschnitt der Zellmasse bestimmt und aus der Masszahl die Wurzel gezogen.

d) *Querschnitt durch das Zwischenhirn.*

Für eine Vergleichsmessung eignet sich am besten diejenige Stelle, wo das Infundibulum vom eigentlichen Ventrikel abzweigt. Die Messung wurde in gleicher Weise durchgeführt wie beim Vorderhirn.

e) *Das Volumen des Riechepithels.*

Dieses wurde mit Hilfe einer Annäherungsformel ermittelt. Zuerst wurde in ähnlicher Weise wie bei (a) der grösste Querschnitt bestimmt, gezeichnet und planimetriert. Die Länge des Organs wurde durch Auszählen der Schnitte festgestellt (Schnittdicke: 10 μ). Die Formel zur Berechnung des Inhalts wurde in Analogie zur Volumenformel des Ellipsoids gefunden:

$$V = \frac{4}{3} \cdot \pi \cdot a \cdot b \cdot c ,$$

wobei a, b, c die halben Achsen des Ellipsoids sind.

$\pi \cdot a \cdot b$ ist der Inhalt der Ellipsenfläche. Diese wird in der Formel ersetzt durch die gemessene Fläche F. Die Annäherungsformel lautet daher:

$$V = \frac{4}{3} \cdot F \cdot \frac{L}{2} ,$$

wobei F die gemessene Fläche des grössten Querschnittes, L die durch Auszählen der Schnitte festgestellte Länge des Organs ist. Weil F in cm² bei Zeichenapparat-Vergrösserung von 220 ×, L aber in μ gemessen wurde, kommt noch ein weiterer Faktor in die Formel. Dieser dient dazu, die in cm² bei einer Vergrösserung von 220 × gemessene Fläche so umzurechnen, dass die Volumenberechnung die Dimension μ^3 hat. Aus den gleichen Gründen wie bei (*b*) war es nötig, die Werte in ein lineares Mass umzurechnen.

f) *Das Volumen des Ohres.*

Das Ohrvolumen wurde in gleicher Weise wie das Volumen der Nase (Riechepithel) gemessen und berechnet.

Bei allen Messungen, die mit dem Zeichenapparat ausgeführt wurden, betrug die Vergrösserung 48 ×, wenn am Lebenden gemessen wurde, und 220 ×, wenn mikroskopische Schnitte untersucht wurden.

III. DAS NORMALE WACHSTUM DER KOPFORGANE.

Die Organentwicklung ist bei späteren Stadien von *Triton alpestris*-Larven verhältnismässig wenig bekannt. Bevor die durch Operationen hervorgerufenen

VOL. 31 1950

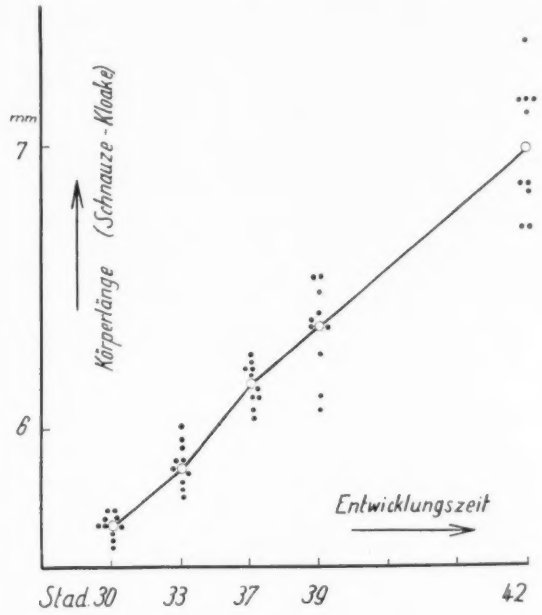


Abb. 2. Längenwachstum des ganzen Körpers beim normalen Keim, gemessen von der Schnauzenspitze bis zur Kloake.

Missbildungen und Abnormalitäten analysiert werden konnten, musste das normale Wachstum der betreffenden Organe näher untersucht werden.

1) Wachstum und Wachstumskurven.

Es war vor allem wichtig, das Längenwachstum des ganzen Körpers zu verfolgen. Als Messtrecke eignet sich für diesen Zweck am besten die Strecke Schnauzenspitze — Kloake, da die Schwanzlänge sehr variabel ist. In Abb. 2 ist das Körperwachstum graphisch dargestellt. Die Verbindungskurve der Mittelwerte bildet eine Gerade. Ein solcher Verlauf der Kurve ergibt sich nur, wenn während der Beobachtungszeit die Temperatur konstant gehalten wird.

Da die Körperlänge linear mit der Entwicklungszeit zunimmt, ist es bei den folgenden Untersuchungen unwichtig, ob man auf der Abszisse das in Abb. 2 dargestellte Längenwachstum der Kontrollen oder die Zeit abträgt.

Abb. 3 zeigt den Verlauf des Augenwachstums. Am Anfang, zwischen den Stadien 27 und 30, verläuft das Wachstum mässig schnell, um sich dann zu beschleunigen und nach dem Gl.-Stad. 39 wieder zu verzögern. Wir haben eine fast ideale Wachstumskurve vor uns, die — nach SIMPSON and ROE (1939) mit ihrem steilsten Teil in der Mitte — S-förmig verläuft. Die Verzögerung der Wachstumsgeschwindigkeit nach dem Gl.-Stad. 39 kann aber

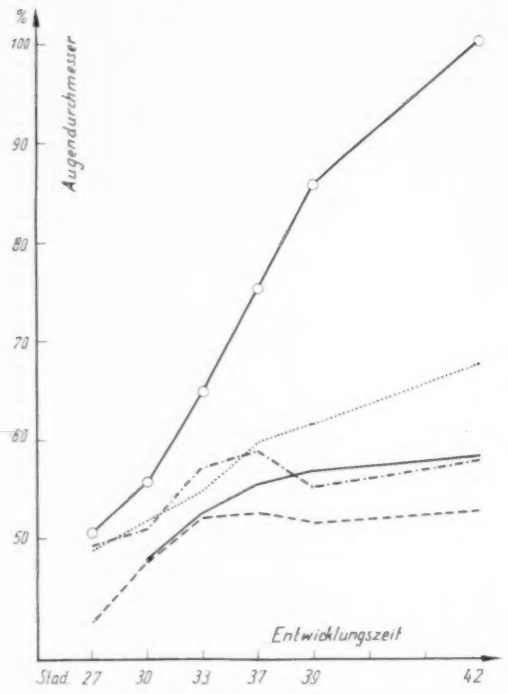


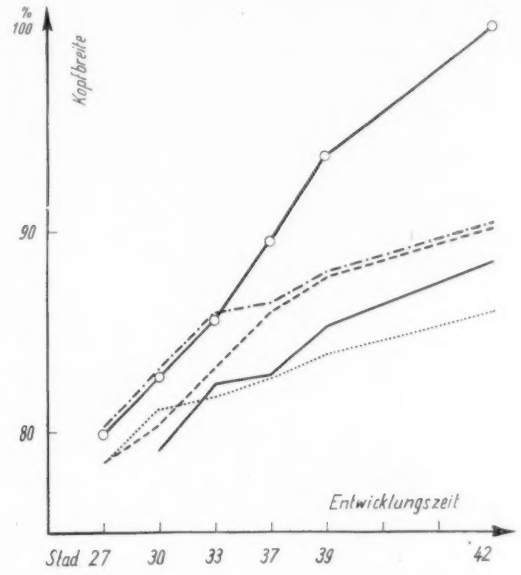
Abb. 3. Wachstum des Augendurchmessers. (Mittelwertskurve; Anzahl der Fälle in Tab. 4.) -o——o- Kontrollen, - - - - - Isolierte Kopfstücke der Serie A, - - - - - - - Isolierte Kopfstücke der Serie B, ... Isolierte Kopfstücke der Serie C, ——— Zirkulationslose Keime der Serie D. Normale Augengrösse im Gl.-Stad. 42 = 100 %.

nicht davon herrühren, dass die Adultgrösse erreicht würde, sondern die Ursache dafür liegt wohl darin, dass die Larven vor der ersten äusseren Nahrungsaufnahme eine kurze Periode des Hungerns durchmachen.

Eine ganz entsprechende Form zeigt die Wachstumskurve der Kopfbreite (Linsendistanz), die ebenfalls an lebenden Larven gemessen wurde. (Abb. 4.)

Die übrigen Kurven (Abb. 5, 12, 13, 16, 17) konnten nur auf Grund von Messungen an mikroskopischen Schnitten, also an fixiertem Material festgestellt werden. Allen Kurven ist die geringere Steilheit nach dem Gl.-Stad. 39 gemeinsam, auf deren Ursachen ich schon hingewiesen habe. Im einzelnen zeigen die Kurven Schwankungen der Wachstumsgeschwindigkeit, worauf ich weiter unten noch zurückkommen werde. Eine Ausnahme hievon bilden die Kurven von Nase und Ohr. Dort scheint das Wachstum im Sinne einer Potentialfunktion zu verlaufen. (Abb. 18, 19.)

Damit man die verschiedenen Wachstumskurven miteinander vergleichen kann, mussten die Ordinatenwerte prozentual abgetragen werden. Der End-



Ab. 4. Zunahme der Kopfbreite (Linsenabstand). (Mittelwertskurve; Anzahl der Fälle in Tab. 4.) -o——o- Kontrollen, - - - - - Isolierte Kopfstücke der Serie A, - - - - - - - Isolierte Kopfstücke der Serie B, ... Isolierte Kopfstücke der Serie C, ——— Zirkulationslose Keime der Serie D. 100 % = Normale Kopfbreite im Gl.-Stad. 42.

wert der Messungen im Gl.-Stad. 42 wird bei jedem Organ auf 100 % festgesetzt. Die Messungswerte in den Stadien 27, 30, 33, 37 und 39 werden in Prozenten der Endgrösse ausgedrückt. Die Kurven behalten dabei ihre Form und können nach dieser Umrechnung direkt miteinander verglichen werden. (Tab. 1, Abb. 5.) Die Umrechnungsformel dafür lautet:

$$Y\,\% = \frac{y}{y_n} \cdot 100\,\%\,,$$

wobei y = Grösse des Organs im betr. Stadium,
y_n = Grösse des Organs im Endstadium (Gl.-Stad. 42) ist.

TABELLE 1. „Prozentwerte" der Organgrössen der Kontrollen in verschiedenen Stadien. Organgrösse im Gl.-Stad. 42 = 100 %

Gl.-Stadium	27	30	33	37	39	42
Augendurchmesser	51	56	65	75	86	100
Kopfbreite	59	65	71	79	87	100
Linsendurchmesser	—	63	70	75	86	100
Retina	—	78	85	88	93	100
Zwischenhirn	—	79	87	91	99	100
Vorderhirn	—	52	65	79	91	100

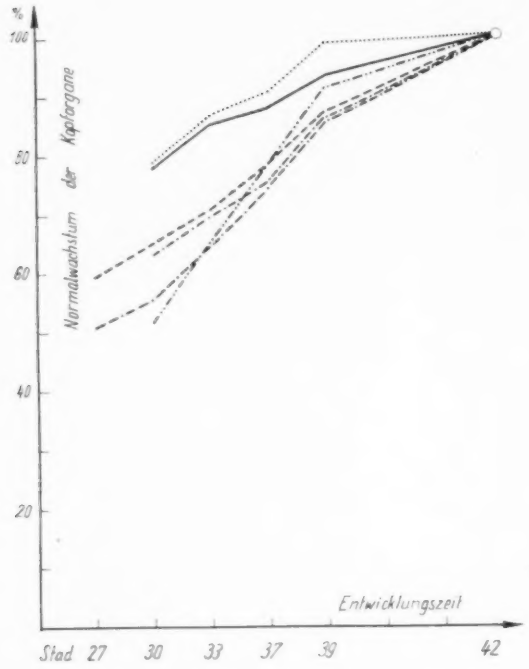


Abb. 5. Wachstumskurven für verschiedene Organe der Kontrollen. (Mittelwertskurve; Anzahl der Fälle in Tab. 4.) Die Werte sind in Prozent angegeben; Organgrösse im Stadium 42 = 100 %. ..... Zwischenhirn, ——— Retina, - - - - - Kopfbreite, - - - - - - - Linse, - - - - - - - - Augendurchmesser, - - - - - - - Vorhirn.

Abb. 5 zeigt, dass die Kurven von Retina und Zwischenhirn wesentlich flacher verlaufen als die übrigen Kurven. Am steilsten steigt die Kurve für das Vorderhirn. Nirgends ist eine Änderung in der Wachstumsgeschwindigkeit im Zeitpunkt des Beginns der Blutzirkulation festzustellen (Gl.-Stad. 30, Abb. 1).

2) Die Wachstumsgeschwindigkeit.

Aus der Darstellung der Wachstumskurven (Abb. 5) der einzelnen Organe geht hervor, dass die Wachstumsgeschwindigkeit während der Entwicklung wechselt. Die Wachstumsgeschwindigkeit ist nach SIMPSON and ROE (1939) als Zunahme der Organgrösse pro Zeit definiert:

$$v = \frac{y_{t2} - y_{t1}}{t_2 - t_1}$$

wobei y_t = Grösse des Organs zum Zeitpunkt t_1, resp. t_2.
t_1, t_2 = Zeitpunkt der ersten, resp. zweiten Messung.

Damit die Wachstumsraten der verschiedenen Organe miteinander verglichen

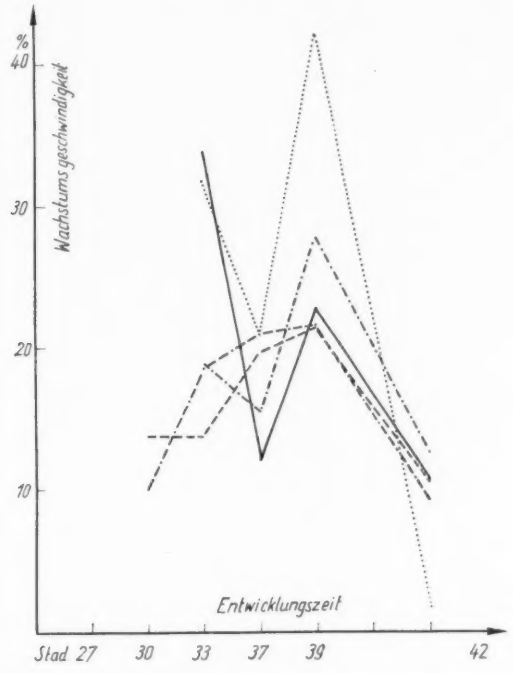


Abb. 6. Arithmetische Wachstumsgeschwindigkeit für verschiedene Organe der Kontrollen. (Mittelwertskurve; Anzahl der Fälle in Tab. 4.) ..... Zwischenhirn, ——— Retina, - - - - - Kopfbreite, - · - · - · Linse, - - - - - - - Augendurchmesser.

werden können, ist es vorteilhaft, wenn man den Zuwachs zwischen zwei Messungen auf den ganzen Zuwachs bezieht.

Die Formel für die ganze Berechnung lautet dann:

$$2v = 2\,\frac{y_{t2} - y_{t1}}{(yt_n - yt_o)\cdot t}\cdot 100\,\%$$

yt_1 = Organgrösse bei der ersten Messung.
yt_2 = Organgrösse bei der zweiten Messung.
yt_o = Organgrösse am Anfang der Beobachtung.
yt_n = Organgrösse im Gl.-Stad. 42.
t = Zeitintervall zwischen zwei Messungen in Tagen.

Der konstante Faktor 2 empfiehlt sich aus praktischen Gründen, weil die Messintervalle meist zwei Tage betragen.

Abb. 6 zeigt die Kurven dieser prozentualen, arithmetischen Wachstumsraten. Die Kurven für Zwischenhirn, Retina und Linsendurchmesser zeigen denselben Wachstumsrhythmus, während die Kurve für den Augendurchmesser einen anderen Verlauf nimmt.

IV. EXPERIMENTE.

1) Isolierte Kopfstücke.

Das Wachstum der Kopforgane wurde nicht nur in der Normalentwicklung verfolgt, sondern auch unter abnormalen Bedingungen näher untersucht. Solche wurden durch Isolierung von Kopfstücken geschaffen, indem Keime im Gl.-Stad. 22/23 an verschiedenen Stellen quer durchschnitten wurden, wie in Abb. 7 dargestellt ist. Die beiden Teile, das Kopfstück und das Schwanzstück wurden separat weitergezüchtet.

Die Kopfstücke entwickeln sich vorerst normal weiter. Bis zum Gl.-Stad. 30 lässt sich kein Unterschied gegenüber den Kontrollen feststellen. Das Körperpigment erscheint normal und gleichzeitig wie bei den Kontrollen, also im Gl.-Stad. 27 (Abb. 1), durchschnittlich zwei Tage nach der Operation. Auch die Entwicklung von Balancer und Augenblasen verläuft zuerst normal. Die Kiemen fehlen bei Serie A (Abb. 7) gänzlich, da ihre Anlagen hinter der Schnittstelle liegen. Bei Serie B bleibt ihre Entwicklung vom Gl.-Stad. 30 an hinter den Kontrollen zurück, und die Kiemen bleiben stummelförmig. Etwas weiter läuft die Entwicklung bei Serie C, wo auch Seitenäste entwickelt werden, ohne dass sie jedoch Grösse und Differenzierungsgrad von normalen Kiemen erreichen, was schon DRASTICH (1925) gezeigt hat. Etwa 3—4 Tage nach der Operation, d. h. zu einem Zeitpunkt, in dem die Kontrollen das Stadium 30 erreicht haben, beginnt in den Kopfstücken der Serien B und C das Herz zu pulsieren, dessen Blastem rostral von der Schnittebene lag. Die Kontraktionen des schlauchförmigen Herzens sind unregelmässig und langsamer als bei den Kontrollen. Da die Kopfstücke an ihrem Hinterende oft blasig aufgetrieben sind, lassen sich die Herzkontraktionen gut verfolgen. Nach REAGAN (1917) und SLONIMSKY (1931) liegt das Erythrocytenblastem bei den Serien A und B ganz, bei Serie C teilweise hinter dem Schnitt. Daher sind diese im Kreislaufsystem der Tiere der Serien A und B gar nicht, bei Serie C nur in beschränktem Masse festzustellen, was vor allem in den Kiemen möglich ist. An den Individuen der Serie C entwickeln sich auch vordere Extremitäten. Ihr Wachstum verläuft langsamer als normal. Während die Kontrolltiere im Gl.-Stad. 42 (Abschluss der Entwicklungsbeobachtung) an der vorderen Extremität drei Zehen ausgebildet haben, weisen die Kopfstücke nur deren zwei auf. Die auffälligste Abweichung in der Weiterentwicklung ist das geringere Wachstum der Augen, das zu einer typischen Mikrophthalmie führt. Ausserdem ist die Bildung der Pigmente, sowohl des schwarzen Aderhautpigmentes wie des goldenen Irispigmentes, verzögert.

Das Schwanzstück entwickelt sich ebenfalls zuerst normal; später zeigt sich eine ventrale Abbiegung, deren Genese von HADORN (1947) eingehend analysiert wurde.

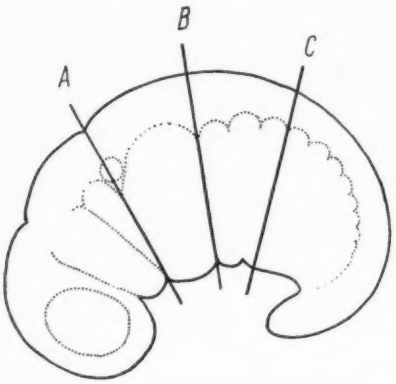


Abb. 7. Operationsschema für Isolierung von Kopfstücken. *A* Schnitt auf Höhe des Gehörbläschens, *B* Schnitt zwischen Kiemen- und Vornierenwulst, *C* Schnitt drei Myotome hinter Schnitt (*B*).

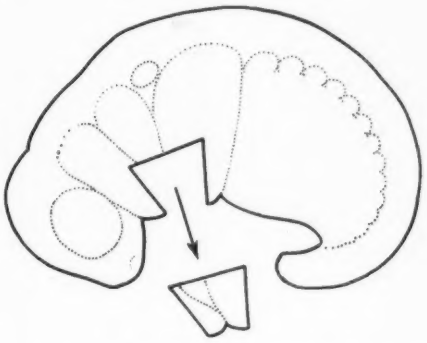


Abb. 8. Exstirpation des Herzblastems im Stadium 24.

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2) Exstirpation des Herzblastems. (Serie D.)

Bei Keimen im Gl.-Stad. 24 (Abb. 8) wurde das Herzblastem exstirpiert. Durch diesen viel kleineren Eingriff wird erreicht, dass die Blutzirkulation im Kopf ebenfalls ausfällt. Diese Operation ist mit Hilfe eines kleinen Messers leicht auszuführen. Um sauberer schneiden zu können, wird sie an Keimen ausgeführt, die einen Tag älter sind, als diejenigen, die entzweigeschnitten wurden. In diesem Stadium (Gl.-Stad. 24) sind die Keime schon ein wenig gestreckt.

In der Weiterentwicklung erscheinen Körperpigment und Balancer normal und gleichzeitig wie bei den Kontrollen. Die Kiemen werden ausgebildet, bleiben aber stummelförmig, oder weisen nur einen Seitenast auf. Das Erythrocytenblastem verbleibt bei diesem Experiment am operierten Tier, doch kann das Blut nicht zirkulieren, da das Herz fehlt. Die Entwicklung der Vorderextremität bleibt in ähnlichem Ausmass wie bei Serie C zurück (S. 200, sowie Abb. 9 und 10). Bei Abbruch der Beobachtungen (Gl.-Stad. 42 der Kontrolle) sind bei den zirkulationslosen Keimen zwei Zehen differenziert. Im gleichen Zeitpunkt haben indessen die Kontrollen drei Zehen an der Vorderextremität. Der Schwanz entwickelt sich normal; die Tiere schwimmen herum und verhalten sich wie die Kontrollen.

Bei dieser Serie wurde in jedem einzelnen Fall geprüft, ob die Zirkulation gänzlich ausfiel. Tiere mit nur unvollständigem Zirkulationsausfall wurden ausgeschieden.

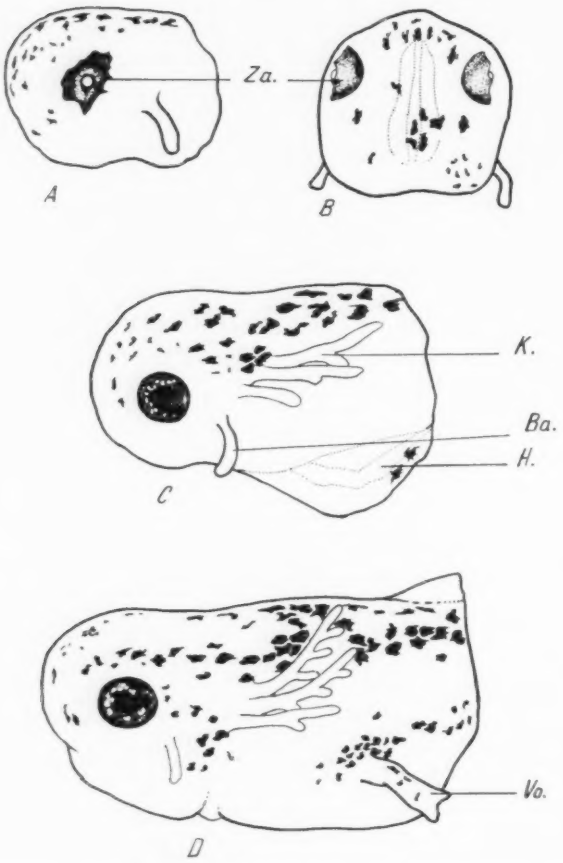


Abb. 9. Verschiedene Kopfstücke im Endstadium der Beobachtung (Gl.-Stad. 42 der Kontrollen). A und B: Kopfstücke der Serie A mit „Zackenaugen" (*Za.*). C: Kopfstück der Serie B. (*K.*) Kiemen, (*Ba.*) Balancer, (*H.*) Herz. D: Kopfstück der Serie C. (*Vo.*) Vorderextremität.

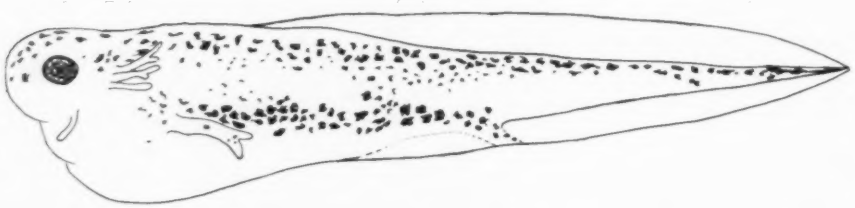


Abb. 10. Zirkulationsloser Keim der Serie D im Endstadium der Beobachtung.

3) Vergleich der Ergebnisse.

a) *Augen.*

Die erste auffallende Abweichung von der Normalentwicklung besteht in der verzögerten Bildung der Augenpigmente. Diese Verzögerung ist bei den Serien A, B und D ungefähr gleich stark, bei Serie C (mit geringer Blutzirkulation) etwas schwächer. Alle Kontrollen haben im Gl.-Stad. 37 schwarze Augen, in denen sich goldgelbes Irispigment ausbreitet, was an über 30 Tieren festgestellt wurde. Im gleichen Zeitpunkt kann man nur bei einem Teil der Kopfstücke und zirkulationslosen Keime Irispigment beobachten. (Tab. 2.) Während 96 % der Kontrollen Irispigment in den Augen zeigen,

TABELLE 2. Entwicklungsgrad der Augenpigmente bei operierten Tieren und Kontrollen zu einem Zeitpunkt, da die Kontrollen das Gl.-Stad. 37 erreicht haben.

Serie	A	B	C	D	Kontrollen
Zahl der Fälle	20	25	26	23	56
Nur Anfangsstadien für schwarzes Pigment	13	14	3	4	—
Auge ganz schwarz	6	9	14	10	2
Bereits Anfangsstadien für Irispigment . .	1	2	9	9	54

ist im gleichen Zeitpunkt (Gl.-Stad. 37) die Augenentwicklung nur bei 5 % der Tiere von Serie A, bei 8 % von Serie B, bei 35 % von Serie C und bei 39 % von Serie D so weit fortgeschritten.

Zusammenfassend kann man sagen, dass das schwarze Pigment bei den operierten Tieren durchschnittlich um 2 Tage, das goldgelbe Irispigment um 5 bis 6 Tage verzögert erscheint. (Abb. 11.)

In der Weiterentwicklung der Augen zeigt sich bald, dass sich das Augenwachstum verlangsamt, sodass es zur Mikrophthalmie kommt. Ausserdem nehmen bei Larven vom Gl.-Stad. 37 die Augen vielfach eine abnormale Form an; es kommt zur Bildung von „Zackenaugen" (HADORN 1945). An Stelle eines runden Augenbechers entsteht ein unregelmässig geformtes Auge, dessen Rand zackig erscheint (Abb. 9). Die Häufigkeit der Zackenaugen ist bei den einzelnen Versuchsserien verschieden (Tab. 3). Der prozentuale Anteil von Individuen mit Zackenaugen ist umso grösser, je näher die Schnittstelle bei den Augen liegt. Der sehr geringe Anteil von Zackenaugen bei Serie C mag durch minimale Blutzirkulation mitbedingt sein. Bei Serie D, wo die Blutzirkulation fehlt, ohne dass eine so grosse Wunde wie bei den anderen Serien entsteht, ist der Anteil an Zackenaugen ebenfalls gering.

Eine Messung des Augendurchmessers in verschiedenen Stadien zeigt, dass die Augen der operierten Individuen im Wachstum hinter der Normalgrösse zurückbleiben. In Abb. 3 sind die Wachstumskurven für die Augen

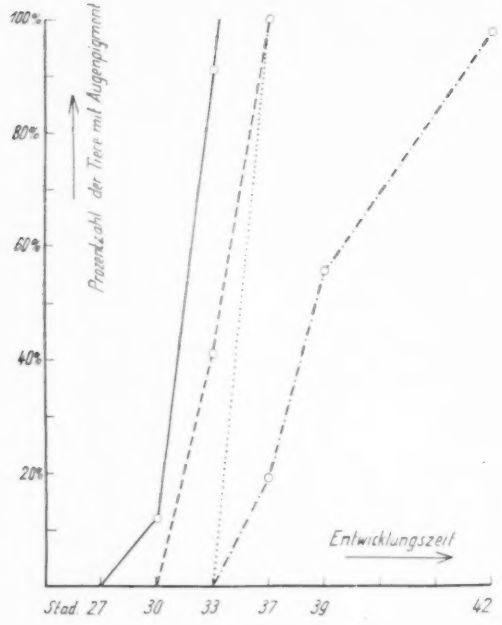


Abb. 11. Entwicklung der Augenpigmente. (Anzahl der Fälle in Tab. 2.) -o——o- Schwarzes Augenpigment bei den Kontrollen, -o---o- Schwarzes Augenpigment bei Kopfstücken und zirkulationslosen Keimen, .o....o. Goldgelbes Irispigment bei den Kontrollen, -o-----o- Goldgelbes Irispigment bei Kopfstücken und zirkulationslosen Keimen.

der Tiere der einzelnen Versuchsserien dargestellt und mit der normalen Wachstumskurve vergleichbar. Bei den Serien A, B und D kommt es zu einem Wachstumsstillstand; bei Serie C schreitet das Wachstum fort, aber langsamer als normal. Bei allen Kopfstücken und zirkulationslosen Keimen ist aber die Augengrösse zu einer Zeit, da die Kontrollen das Gl.-Stad. 42 erreicht haben, geringer als bei den letzteren (Tab. 3).

Interessant ist das Verhalten der einzelnen Augenteile. (Linse und Retina). Wie HARRISON (1929) gezeigt hat, sind in kleineren Augen auch die einzelnen Augenteile kleiner; aber die Wachstumshemmung ist für die verschiedenen Teile verschieden stark (Tab. 3). Die Wachstumskurven in Abb. 12 und 13 zeigen, dass das Wachstum von Linse und Retina bei isolierten Kopfstücken und zirkulationslosen Keimen blockiert wird. Eine Ausnahme davon macht nur das Linsenwachstum bei Serie C, was damit zusammenhängt, dass diese Kopfstücke eine geringe Blutzirkulation aufweisen. Da in jedem Stadium eine Anzahl Tiere fixiert wurde, war es nicht möglich, immer die gleichen Tiere zu messen, sodass dadurch individuelle Grössenunterschiede ins Gewicht fallen. Fallende Abschnitte der Kurven sind daher durch Zufall bedingt, was statistisch geprüft wurde.

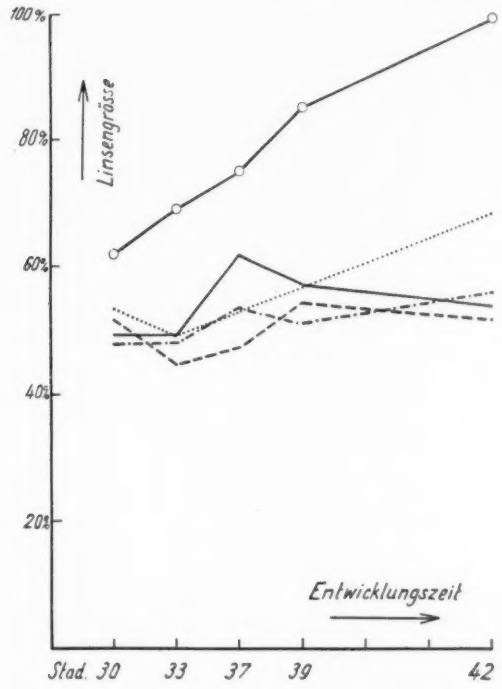


Abb. 12. Wachstum der Linse (Linsendurchmesser). (Mittelwertekurve; Anzahl der Fälle in Tab. 4.) -o——o- Kontrollen, - - - - - Isolierte Kopfstücke der Serie A, - - - - - - Isolierte Kopfstücke der Serie B, ... Isolierte Kopfstücke der Serie C, ——— Zirkulationslose Keime der Serie D. 100 % = Linsengrösse der Kontrollen im Gl.-Stad. 42.

Die Tabelle 3 zeigt, dass für das Ausmass der Wachstumshemmung nicht so sehr die Art des Eingriffs (Zerschneiden oder Exstirpation des Herzblastems) entscheidend ist, als vielmehr die Tatsache, ob eine Larve Blutzirkulation aufweist oder nicht. Während die Werte der Serien A, B und D meist recht nahe beieinander liegen, weichen die Werte der Serie C (geringe Blutzirkulation) oft beträchtlich von ersteren ab.

Für die Retina wurde die Wachstumshemmung näher untersucht. Während der lineare Wert des Retinaquerschnittes (S. 193) bei der Kontrolle grösser ist als beim operierten Tier, zeigt sich in Bezug auf die Dicke der Retina das Umgekehrte. Bei der normalen Entwicklung der Retina nimmt die Dicke bis zum Gl.-Stad. 37 zu; nachher folgt eine Phase der Ausbreitung; die Dicke der Retina nimmt ab. Beim Auge des operierten Tieres nimmt die Dicke der Retina bis zum Gl.-Stad. 42 zu. Man kann das so deuten, dass wegen des gehemmten Gesamtwachstums des Auges die flächige Ausbreitung der Retina verhindert ist.

TABELLE 3. Prozentwerte der Organgrössen im Gl.-Stad. 42 der Kontrollen. Alle Werte sind linear angegeben. Die eingeklammerten Zahlen geben die Anzahl der Fälle an. Das Ohrvolumen ist in dieser Zusammenstellung bei Serie A nicht aufgeführt, da das Ohr bei den Kopfstücken dieser Serie infolge der Operation meist fehlt.

Serie	A	B	C	D	Kontrollen
Prozentualer Anteil von „Zackenaugen"	96,4 (28)	53,6 (28)	4.4 (28)	10,3 (29)	0,0 (76)
Augendurchmesser	52,3 (20) ± 1,7	57,5 (20) ± 1,4	67,2 (15) ± 2,9	58,6 (20) ± 1,1	100,0 (76) ± 0,6
Linsendistanz (Kopfbreite)	79,8 (20) ± 1,9	80,3 (20) ± 2,1	71,7 (19) ± 2,0	76,6 (18) ± 1,7	100,0 (73) ± 0,7
Linsendurchmesser	52,3 (18) ± 2,0	56,3 (11) ± 2,3	68,8 (16) ± 3,0	54,8 (16) ± 1,8	100,0 (30) ± 1,3
Retina-Querschnitt	75,0 (18) ± 2,9	79,0 (11) ± 4,0	79,7 (13) ± 1,8	79,7 (16) ± 1,3	100,0 (30) ± 0,9
Vorderhirn-Querschnitt	40,7 (12) ± 2,1	50,8 (8) ± 3,4	73,4 (14) ± 2,7	68,9 (14) ± 1,8	100,0 (30) ± 1,2
Zwischenhirn-Querschnitt	65,0 (14) ± 0,9	76,3 (12) ± 2,0	85,2 (16) ± 1,9	83,8 (15) ± 2,0	100,0 (30) ± 0,8
Nasenvolumen	60,3 (15) ± 1,4	58,8 (10) ± 1,1	72,2 (13) ± 1,5	69,6 (12) ± 1,4	100,0 (30) ± 1,7
Ohrvolumen	—	60,2 (9) ± 1,5	77,4 (15) ± 1,5	87,5 (13) ± 1,5	100,0 (30) ± 1,6

VOL. 31 1950

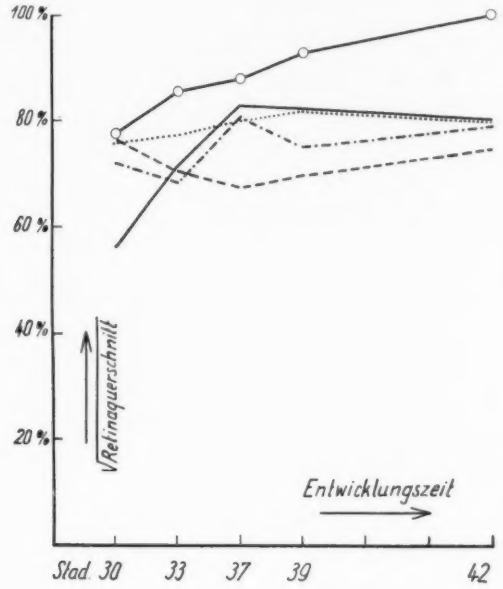


Abb. 13. Wachstum der Retina (Wurzel aus dem Querschnitt). (Mittelwertskurve; Anzahl der Fälle in Tab. 4.) -o——o- Kontrollen, - - - - - Isolierte Kopfstücke der Serie A, - - - - - - - Isolierte Kopfstücke der Serie B, ... Isolierte Kopfstücke der Serie C, ——— Zirkulationslose Keime der Serie D. 100 % = Retina der Kontrollen im Gl.-Stad. 42.

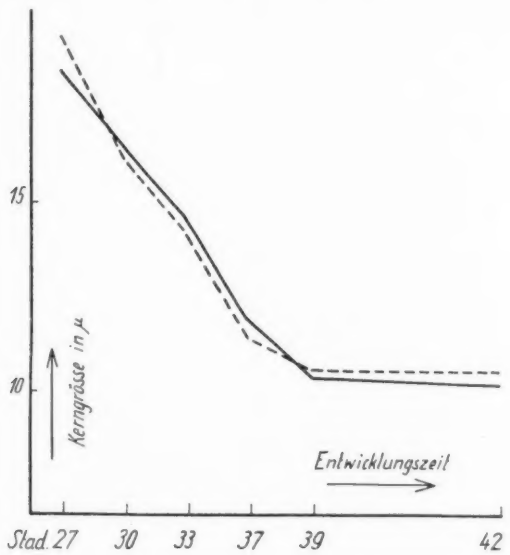


Abb. 14. Abnahme der Kerngrösse (Länge der Kerne). (Mittelwertskurve für je 50 Fälle.) ——— Kopfstücke, - - - - - Kontrollen.

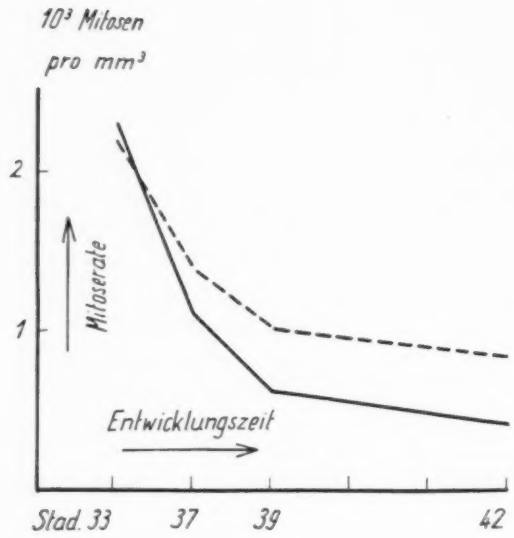


Abb. 15. Abnahme der Mitoserate in der Retina. Zur Ermittlung eines Kurvenpunktes wurden die Mitosen in der Retina von je drei verschiedenen Tieren ausgezählt. ——— Kopfstücke, - - - - - Kontrollen.

Bei einer eingehenderen Untersuchung stellt sich die Frage nach der Kerngrösse und der Kerndichte. Eine Messung in den verschiedenen Stadien zeigt, dass die Kerngrösse bei den Kontrollen wie bei den operierten Tieren in gleichem Masse abnimmt. (Abb. 14.) In der Kerndichte lässt sich im Gl.-Stad. 42 kein Unterschied zwischen operierten Tieren und Kontrollen nachweisen. Daraus folgt, dass die Zahl der Zellen im kleineren Organ eines operierten Tieres geringer sein muss als bei den Kontrollen. Daher ist zu erwarten, dass die Mitoserate bei den Kopfstücken geringer sein muss als bei den Kontrollen. Eine Auszählung der Mitosen in der Retina bezogen auf das Volumen zeigt, dass dies tatsächlich der Fall ist (Abb. 15).

b) *Andere Kopforgane.*

Nachdem die Augenentwicklung bei den Kopfstücken und zirkulationslosen Keimen analysiert, und mit der normalen Entwicklung verglichen worden ist, stellt sich die Frage, ob auch andere Kopforgane von diesem Zirkulationsausfall betroffen worden sind.

Eine vergleichende Messung zeigt, dass die Kopfbreite der operierten Tiere geringer ist als bei den Kontrollen. Allerdings ist hier die Wachstumshemmung weniger stark als bei den Augen. (Abb. 4, Tab. 3.) Merkwürdigerweise ist die Kopfbreite bei den Kopfstücken der Serie C geringer als bei denjenigen der Serien A und B. Dies hängt damit zusammen, dass die

VOL. 31 1950

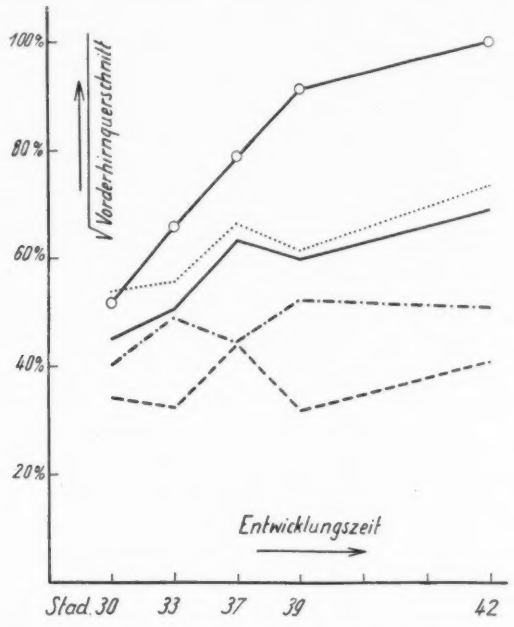


Abb. 16. Wachstum des Vorderhirns (Wurzel aus dem Querschnitt). (Mittelwertskurve; Anzahl der Fälle in Tab. 4.) -o——o- Kontrollen, - - - - - Isolierte Kopfstücke der Serie A, - - - - - - - Isolierte Kopfstücke der Serie B, ... Isolierte Kopfstücke der Serie C, ——— Zirkulationslose Keime der Serie D. 100 % = Grösse des Vorderhirns im Gl.-Stad. 42.

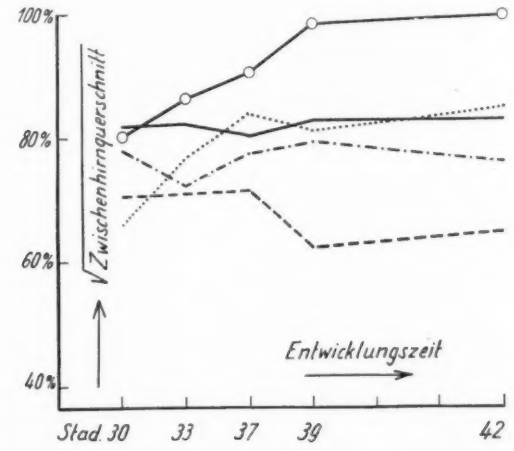


Abb. 17. Wachstum des Zwischenhirns (Wurzel aus dem Querschnitt). (Mittelwertskurve; Anzahl der Fälle in Tab. 4.) -o——o- Kontrollen, - - - - - Isolierte Kopfstücke der Serie A, - - - - - - - Isolierte Kopfstücke der Serie B, ... Isolierte Kopfstücke der Serie C, ——— Zirkulationslose Keime der Serie D. 100 % = Grösse des Zwischenhirns im Gl.-Stad. 42.

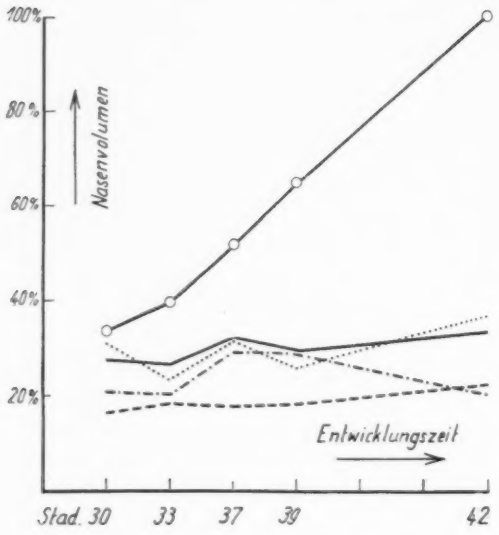


Abb. 18. Zunahme des Nasenvolumens. (Mittelwertskurve; Anzahl der Fälle in Tab. 4.) -o——o- Kontrollen, - - - - - Isolierte Kopfstücke der Serie A, - - - - - - - Isolierte Kopfstücke der Serie B, ... Isolierte Kopfstücke der Serie C, ——— Zirkulationslose Keime der Serie D. 100 % = Nasenvolumen im Gl.-Stad. 42.

Kopfstücke von Serie A und B am Hinterende blasig aufgetrieben sind. Bei der Kopfbreite kommt es nicht zu einem völligen Wachstumsstillstand, sondern nur zu einer Wachstumsverzögerung, was die ständig ansteigenden Kurven der Abb. 4 zeigen.

Die Wachstumskurven von Vorder- und Zwischenhirn verlaufen gleichartig wie beim Auge (Abb. 16, 17). Hier kommt es bei den operierten Tieren ebenfalls zu einem Wachstumsstillstand. Aber die Wachstumshemmung ist beim Vorderhirn relativ stärker als beim Zwischenhirn, was ein Vergleich der Organquerschnitte im Gl.-Stad. 42 der Kontrollen zeigt (Tab. 3).

Wenn die Blutzirkulation fehlt, kommt es bei der Grössenzunahme des Riechepithels wie bei den meisten anderen Organen zu einem völligen Wachstumsstillstand, (Abb. 18, Tab. 3).

Das Wachstum des Hörorganes verläuft anders, worauf schon bei der Diskussion der normalen Wachstumskurven hingewiesen wurde. (S. 196.) Bei den operierten Tieren kommt es hier nicht zu einem Stillstand, sondern nur zu einer Verzögerung des Wachstums. Bei Serie A fehlt das Ohr meist, da der Schnitt auf Höhe des Ohrbläschens ausgeführt wurde. Wie aus Abb. 19 hervorgeht, ist das Wachstum bei Serie B stärker gehemmt als bei den Serien C oder gar D. (Tab. 3.)

VOL. 31 1950

TABELLE 4. Anzahl der Messungen, aus denen die Mittelwerte errechnet und danach die Kurvenpunkte bestimmt wurden.

	Serie	A	B	C	D	Ko	A	B	C	D	Ko	
Augendurchmesser	Stad.											Kopfbreite
	27	22	13	32	—	46	23	10	25	—	34	
	30	28	27	29	19	81	28	17	31	18	43	
	33	29	25	27	24	86	27	24	27	20	79	
	37	27	22	23	18	91	26	23	26	16	82	
	39	28	26	22	14	91	27	28	27	15	91	
	42	20	20	15	20	76	20	20	19	18	73	
Linse	30	3	4	8	5	10	3	5	7	5	10	Retina
	33	6	4	6	6	10	5	4	5	6	10	
	37	5	5	7	5	10	5	5	7	4	10	
	39	4	6	6	7	10	4	7	6	7	10	
	42	18	11	16	16	30	18	11	13	16	30	
Vorderhirn	30	1	1	7	3	8	2	3	3	5	6	Zwischenhirn
	33	5	2	3	6	10	5	3	5	6	10	
	37	2	4	5	4	10	2	5	7	4	10	
	39	1	4	4	6	10	1	5	4	7	10	
	42	12	8	14	14	30	14	12	16	15	30	
Riechepithel	30	3	3	6	5	10	1	2	5	5	6	Ohr
	33	6	4	5	6	10	3	4	5	6	10	
	37	3	5	7	4	10	2	4	7	4	10	
	39	2	5	4	5	10	1	4	5	6	10	
	42	15	10	13	12	30	4	9	15	13	30	

c) *Vergleich der Wachstumskurven.*

Das Wachstum der Kopforgane ist bei allen zirkulationslosen Keimen und Kopfstücken gehemmt. Nun stellt sich die Frage, ob es sich um eine gleichmässige, generelle Hemmung handelt, oder ob die Hemmung für jedes Organ spezifisch ist.

Über diese Frage geben uns ein Vergleich der Wachstumskurven der verschiedenen Organe sowie eine Zusammenstellung der Organgrössen im Gl.-Stad. 42 der Kontrollen Auskunft. (Abb. 20, Tab. 3.) Aus Abb. 20 können wir zwei Tatsachen von Bedeutung herauslesen:

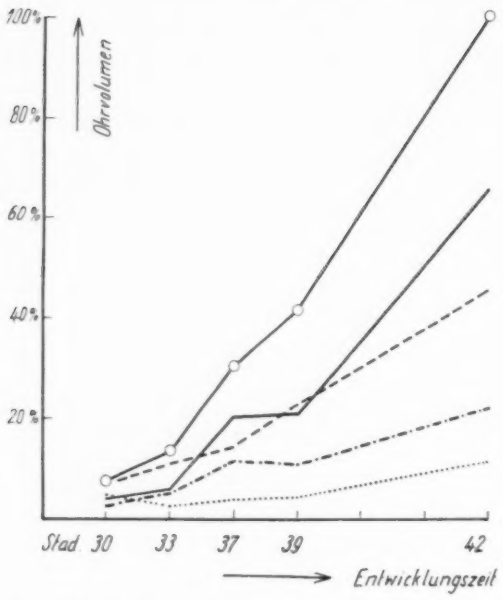


Abb. 19. Zunahme des Ohrvolumens. (Mittelwertskurve; Anzahl der Fälle in Tab. 4.) -o——o- Kontrollen, ... Isolierte Kopfstücke der Serie A, - - - - - - - Isolierte Kopfstücke der Serie B, - - - - - Isolierte Kopfstücke der Serie C, ——— Zirkulationslose Keime der Serie D. 100 % = Ohrvolumen der Kontrollen im Gl.-Stad. 42.

1) Diese Kurven teilen sich in zwei Gruppen auf. Bei der ersten Gruppe liegt die Grösse der Organe im Stadium 42 zwischen 75 und 80 % der Normalgrösse. Das ist für das Zwischenhirn, die Retina und die Kopfbreite der Fall. Bei der zweiten Gruppe (Auge, Linse und Vorhirn) betragen die Werte ca. 55 % der Normalgrösse. Für die Konstruktion dieser Kurven wurden die Werte der Serien, bei denen die operierten Tiere kein Blut haben, zusammengezogen (Serien A, B und D).

2) Verfolgen wir den Verlauf der Kurven, so zeigt sich, dass man eigentlich drei Typen unterscheiden muss:

a) Die Kurve steigt auch nach dem Gl.-Stad. 33 noch an, wenn auch weniger steil als normal. Sie steigt bis zum Stadium 42 der Kontrollen, was nur Hemmung, nicht aber Blockierung des Wachstums bedeutet. Beispiele: Kopfbreite, ev. Vorhirn.

b) Die Kurve steigt nach dem Gl.-Stad. 33 noch ein wenig an, dagegen verläuft sie vom Stadium 37 an wagrecht. Von diesem Stadium an ist also das Wachstum gänzlich sistiert.

Beispiele: Retina, Linse und Augendurchmesser.

c) Die Kurve steigt nach dem Gl.-Stad. 30 gar nicht mehr an, sondern verläuft horizontal. Die dabei auftretenden fallenden Stellen der Verbindungs-

VOL. 31 1950

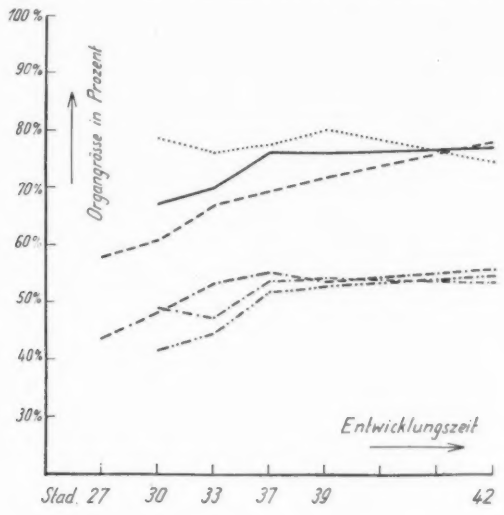


Abb. 20. Wachstumskurven für verschiedene Organe der isolierten Kopfstücke und zirkulationslosen Keime. (Mittelwertskurve; Anzahl der Fälle in Tab. 4; bei dieser Kurve wurden die Fälle der Serien A, B und D zusammengenommen.) ..... Zwischenhirn, ——— Retina, - - - - - Kopfbreite, - - - - - - - - Auge, - - - - - - Linse, - - - - - - - Vorhirn. Organgrösse der Kontrollen im Gl.-Stad. 42 = 100 %.

kurve der Mittelwerte sind unwesentlich und durch Zufall bedingt (S. 204). Das Wachstum ist in diesem Fall schon vom Stadium 30 an blockiert.

Beispiel: Zwischenhirn.

Dieser verschiedene Verlauf der Kurven zeigt, dass nicht alle Organe gleich auf den Zirkulationsausfall reagieren. Die Wachstumshemmung ist organspezifisch, was bereits von HADORN (1945) postuliert wurde.

Zum gleichen Ergebnis führt auch eine Zusammenstellung der Organgrössen im Gl.-Stad. 42, wie sie in Tabelle 3 dargestellt ist.

d) *Zirkulationsausfall als Ursache der Wachstumshemmungen.*

Als Ursache für die Wachstumshemmungen kommt vor allem das Fehlen der Blutzirkulation im Kopf in Frage. Während bei den isolierten Kopfstücken die dorsale Kopfgegend von der Operation betroffen ist, sodass man an eine verhinderte späte Wanderung von Kopforganisatormaterial denken könnte, ist das bei Serie D (Exstirpation des Herzblastems) nicht der Fall. Trotzdem sind hier die Wachstumshemmungen gleich gross. Das Gemeinsame bei allen Serien ist die gestörte oder ganz fehlende Blutzirkulation im Kopf. Dass die Blutzirkulation bei der Bildung der Kopforgane von entscheidender Bedeutung ist, geht auch aus der folgenden Versuchsserie hervor.

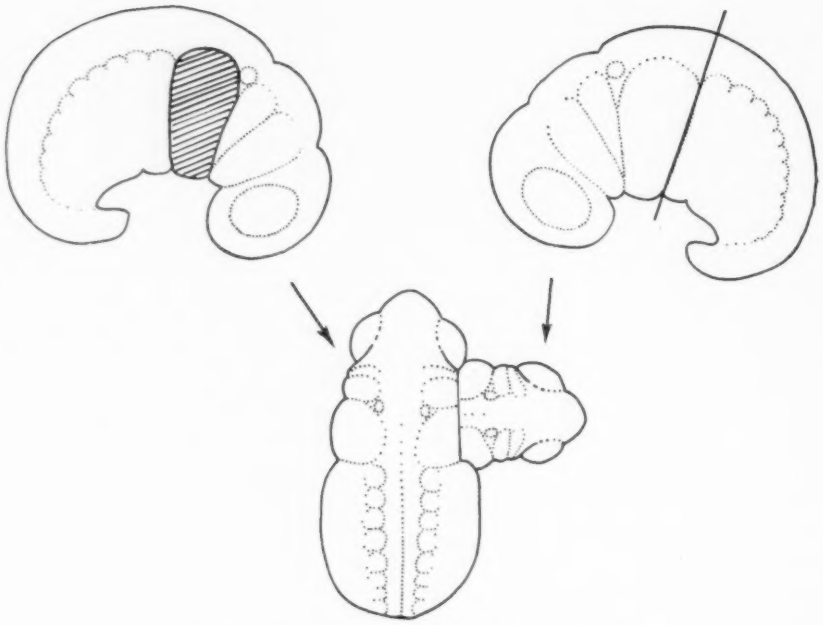


Abb. 21. Operationsschema: „Halbparabiosen".

4) „Halbparabiosen". (Serie H.)

Wie in Serie A, resp. B wurden Kopfstücke hergestellt. Dann wurde einem zweiten Keim die Epidermis in der Kiemengegend abpräpariert und an dieser Stelle ein Kopfstück aufgepropft. (Abb. 21.) So entstand eine „Parabiose" zwischen einem ganzen Keim und einem Kopfstück; ich nenne diese Bildung eine „Halbparabiose". Das Einheilen verlief wie bei einer gewöhnlichen Parabiose sehr rasch. Mit der Zeit entstand eine Larve, die einer Duplicitas anterior nicht unähnlich sah (Abb. 22 A, B).

Physiologisch kommt diese Zusammensetzung einer Bluttransfusion in vivo gleich, indem ein an sich blutloses Kopfstück von einem anderen Tier mit Blut versorgt wird. In der Tat zeigen solche Kopfstücke in den Kiemen die Zirkulation von Erythrocyten. Diese lässt sich gleichzeitig wie bei den Kontrollen erstmals feststellen (Gl.-Stad. 32/33).

In der Weiterentwicklung zeigt sich, dass das Kopfstück je nach dem Grad der Verwachsung Blutzirkulation aufweist oder nicht. Es ergeben sich daher vier Möglichkeiten, die alle realisiert sind:

1) Kopfstück und Träger haben Blut.

2) Nur der Träger hat Blut, während im aufgepfropften Kopfstück keine Zirkulation nachweisbar ist.

VOL. 31 1950

3) Nur das Kopfstück hat Blut, während im Träger keine Zirkulation festgestellt werden kann.

4) Weder im Träger noch im aufgepropften Kopfstück lässt sich Blutzirkulation nachweisen.

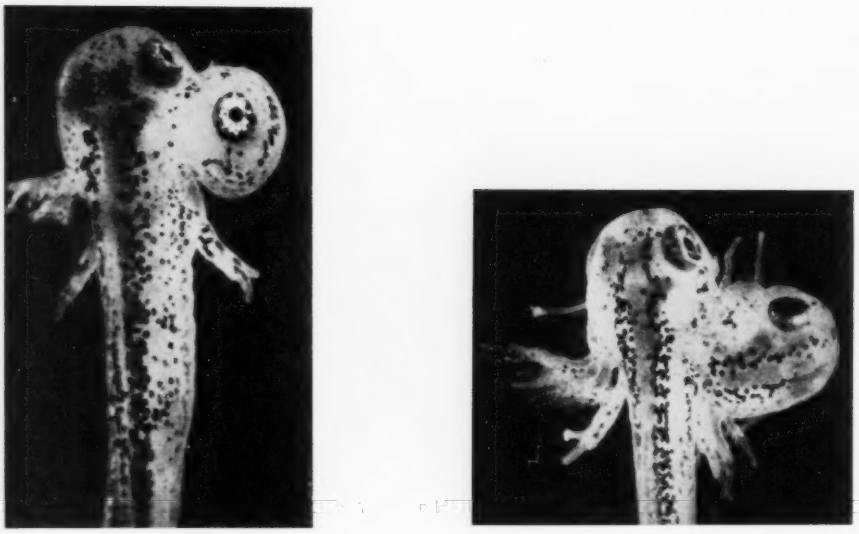
Die Augenpigmente erscheinen in allen durchbluteten Augen gleichzeitig wie bei den Kontrollen, in den undurchbluteten mit der entsprechenden Verzögerung (S. 203), gleichgültig, ob sie sich am Träger oder am angeheilten Kopfstück befinden. Verfolgen wir die Entwicklung der Augen weiter, so zeigt sich, dass Augen an einem durchbluteten Kopf immer grösser sind als Augen an einem nicht durchbluteten Kopf. (Tab. 5.)

TABELLE 5. Prozentwerte der Augengrösse in Serie H. Die Zahlen bedeuten Prozentwerte bezogen auf die normale Augengrösse der Kontrollen im Gl.-Stad. 42. Die eingeklammerten Werte geben Minima und Maxima an.

	Anzahl der Fälle	Mit Blutzirkulation		Ohne Blutzirkulation	
Auge am		Kopfstück	Träger	Kopfstück	Träger
Fall I . . . . . Beide durchblutet .	11	80,2 (69,0—89,1)	88,5 (80,4—94,8)	—	—
Fall II . . . . . Kopfstück ohne, Träger mit Blut .	4	—	97,1 (94,8—97,7)	63,3 (51,7—68,9)	—
Fall III . . . . . Kopfstück mit, Träger ohne Blut	2	87,6 (86,2—89,1)	—	—	64,6 (60,4—68,9)
Fall IV . . . . . Beide ohne Blut .	4	—	—	61,8 (54,6—71,8)	63,2 (57,5—68,9)
Kontrollen	76	—	100,0	—	—
Isoliertes Kopfstück, Serie B .	20	57,5	—	—	—

Die vier in Tabelle 5 zusammengestellten Fälle sollen näher analysiert werden.

Fall I. Die Augen des Kopfstückes sind im Wachstum wenig zurückgeblieben. Sie sind etwas kleiner als die Augen des Trägers, aber bei weitem nicht so klein geblieben wie in Serie B. Der Grössenunterschied zwischen den Augen des Trägers und denjenigen des Kopfstückes ist schwach gesichert ($0{,}01 > P > 0{,}001$). Vielleicht ist durch die Operation die Blutzirkulation beeinträchtigt worden. Wichtiger aber ist die Tatsache, dass sich die gleiche



A B

Abb. 22. „Halbparabiosen" im Endstadium der Beobachtung. Die aufgepfropften Kopfstücke weisen Blutzirkulation auf. A: Kopfstück ohne Kiemen. B: Aufgepfropftes Kopfstück mit Kiemen.

VOL. 31 1950

Blutmenge und der gleiche Dottervorrat, nämlich derjenige des Trägers auf ein grösseres System verteilt. So ist zu erklären, das auch die Augen des Trägers kleiner als normal sind. (Abb. 22 B).

Fall II. Kopfstück und Träger sind nur sehr lose miteinander verbunden, sodass die Blutzirkulation auf den Träger beschränkt bleibt. Hier entwickeln sich die Augen des Trägers normal, während diejenigen des Kopfstückes mikrophthalm werden. Sie haben ungefähr dieselbe Grösse wie in Serie B. Die Abweichung gegenüber den Werten der Augengrösse der Kopfstücke im Fall I ist gut gesichert ($P < 0{,}001$) (Abb. 23).

Fall III. Durch Verletzung bei der Operation kam es vor, dass in zwei Fällen nur der Träger, nicht aber das Kopfstück mikrophthalm wurde. Allerdings sind in diesem Fall die Augen des Kopfstückes kleiner als normal. Die Abweichungen sind infolge der geringen Anzahl von Individuen nicht gesichert.

Fall IV. Sind Träger und Kopfstück nicht durchblutet, d. h. fehlt dem ganzen System die Blutzirkulation, so werden beide Teile mikrophthalm. Auch in diesem Fall ist der Augendurchmesser beim Träger grösser als beim Kopfstück, wobei das Verhältnis der Augengrössen ähnlich ist wie im Fall I.

Zusammenfassung. Das Auge des Kopfstückes bleibt immer kleiner als das Auge des Trägers, gleichgültig, ob beide durchblutet oder beide

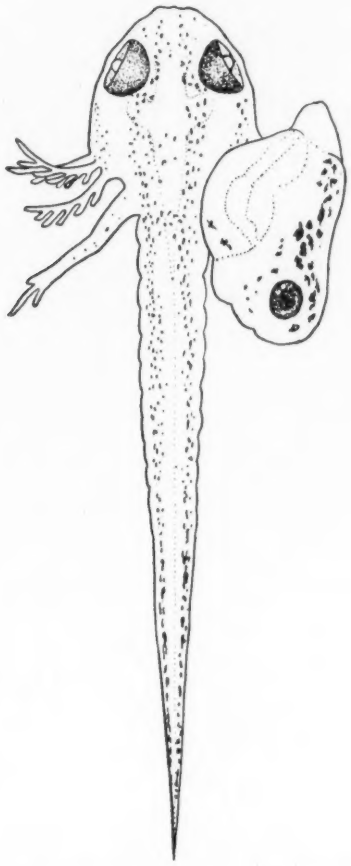


Abb. 23. „Halbparabiosen" im Endstadium der Beobachtung. Das aufgepfropfte Kopfstück zeigt keine Blutzirkulation; es ist mikrophthalm und die Augengrösse vergleichbar mit Serie B.

ohne Blut sind. Der Unterschied ist zwar gering, aber immerhin gesichert. Er rührt wahrscheinlich davon her, dass beim Einheilungsprozess das Kopfstück stärker beeinträchtigt wird als der Träger. Wichtiger ist der sehr gut gesicherte Unterschied ($P < 0{,}001$) in der Augengrösse, der davon abhängt, ob das Auge durchblutet ist oder nicht, gleichgültig, ob es sich am Träger oder am angeheilten Kopfstück befindet. Diese Versuchsserie zeigt den entscheidenden Einfluss, den das Blut auf das Augenwachstum ausübt.

Ganz analoge Verhältnisse ergeben sich, wenn ein kleineres Kopfstück aufgepflanzt wird, das der Serie A entspricht, also vor dem Kiemenwulst geschnitten wurde. Hier fehlt allerdings die Kombination, bei der das Kopf-

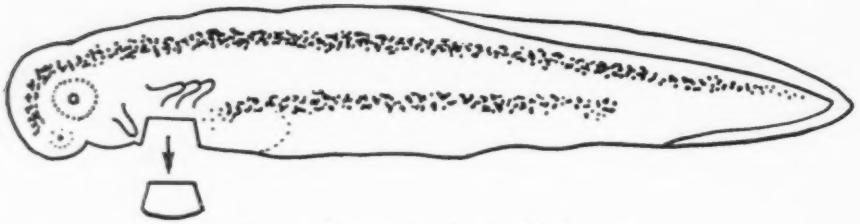


Abb. 24. Herzexstirpation im Stadium 33.

stück, nicht aber der Träger durchblutet wird. Dieser Versuch wurde mit weiteren 15 Tieren durchgeführt. (Abb. 22 A.)

5. Der Einfluss des Blutes als Dauerwirkung.

Nachdem feststeht, dass bei dieser Wachstumshemmung das Blut eine wichtige Rolle spielt, fragt es sich, welcher Art diese Einflüsse sind. Das Blut hat verschiedene Funktionen, so z. B. Sauerstoffversorgung der Organe, Transport von Nährstoffen und Wirkstoffen etc. Bevor wir uns mit diesem Problem befassen, ist festzustellen, ob es sich bei diesem Bluteinfluss um eine Dauerwirkung oder um einen einmaligen Anreiz handelt. Darüber geben die beiden folgenden Versuchsserien Aufschluss.

Serie O.

Bei Larven im Gl.-Stad. 33/34 wird das schon pulsierende Herz exstirpiert. Für diese Operation werden nur Tiere gebraucht, bei denen die Blutzirkulation im Auge gut sichtbar ist. (Abb. 24.)

Die Wunde verheilt rasch, die Vorderextremitäten wachsen aus, entwickeln Zehen; aber die Augen bleiben im Wachstum zurück, es kommt zur Mikrophthalmie. Der Augendurchmesser beträgt am Ende der Beobachtungszeit (Kontrollen im Gl.-Stad. 42) nur 61,2 % der Normalgrösse. Das bedeutet, dass das Augenwachstum vom Zeitpunkt der Augenentwicklung an stillsteht (Abb. 25); wenn die Kontrollen das Gl.-Stad. 42 erreicht haben, sind die Augen der operierten Tiere noch gleich gross wie im Stad. 33, obwohl die gesamte Grösse des Tieres in dieser Zeit erheblich zugenommen hat. Zackenaugen traten bei diesem Versuch nie auf. Das Endergebnis entspricht weitgehend demjenigen von Serie G. Es ist auch statistisch kein Unterschied zwischen den beiden Serien nachzuweisen (t-Test: $P > 0{,}05$). Die Wachstumshemmung, die diese spätbedingte Mikrophthalmie verursacht, tritt also erst in einer Zeit auf, da bei den Kontrollen das Blut im Kopf zirkuliert (nach dem Gl.-Stad. 33). Dieses Experiment ist somit ein weiterer Beweis für die Bedeutung der Blutzirkulation bei der Entstehung der Mikrophthalmie (Tab. 6).

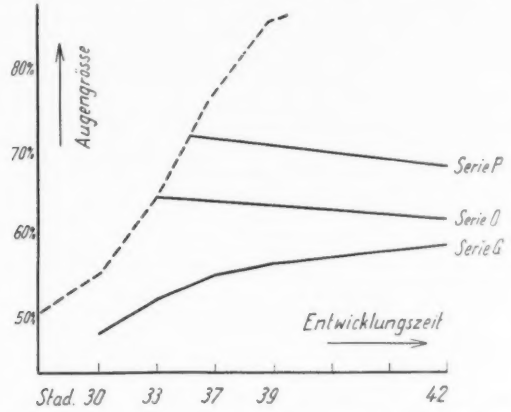


Abb. 25. Augenwachstum bei zirkulationslosen Keimen, deren Herz in den Stadien 33, resp. 34—37 exstirpiert wurde. Augengrösse der Kontrollen im Gl.-Stad. 42 = 100 %.

OL.
31
950

Serie P.

Das pulsierende Herz wird aus Larven der Gl.-Stadien 34—37 exstirpiert. In der Weiterentwicklung tritt wieder Mikrophthalmie auf. Die Augen werden aber, wie erwartet, grösser als bei Serie O. Die Augengrösse beträgt 67,8 % des normalen Augendurchmessers (Tab. 6), wobei wiederum keine Zackenaugen auftreten. (Abb. 25.) Der verschiedenen Stadien wegen, die für den Eingriff verwendet worden sind, ist die Streuung gross; daher ist der Unterschied gegenüber der Serie O nicht eindeutig gesichert ($0{,}01 < P < 0{,}05$), was aber für das Ergebnis unwesentlich ist, da diese beiden Versuchsserien zusammengehören. Für Serie O wurden 11, für Serie P 10 Tiere verwendet.

Es handelt sich beim Bluteinfluss um eine Dauerwirkung und nicht um einen einmaligen Reiz. Wird die Blutzirkulation unterbunden (in diesem Fall durch Herzexstirpation), bleibt das Augenwachstum stehen.

TABELLE 6. Vergleich der Augengrösse in Prozenten (Augengrösse der Kontrollen im Gl.-Stad. 42 = 100 %) bei Herzexstirpation in verschiedenen Stadien.

Serie		D	O	P
	Zahl der Fälle	21	11	10
Zirkulationslose Keime	Herzexstirpation im Gl.-Stad. .	25	33	34—37
	Augendurchmesser im Gl.-Stad. 42 (%)	58,6	62,2	67,8
Kontrollen	Augendurchmesser der Kontrollen (%)	55,4	64,7	69,9
	im Gl.-Stad.	30	33	34—37

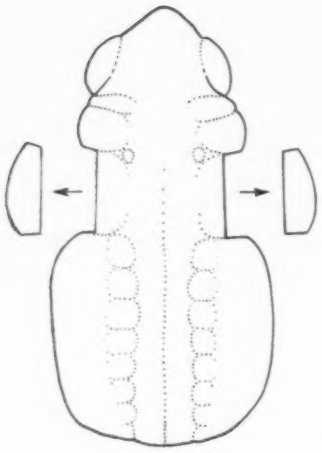


Abb. 26. Exstirpation der Kiemenwülste.

VOL 31 195

6) Ursachen der Mikrophthalmie.

Es stellt sich nun die Frage, ob für diese Wachstumshemmungen in erster Linie mangelnde Sauerstoffzufuhr oder ungenügende Ernährung verantwortlich gemacht werden soll.

a) *Verminderung der Atmung.*

Serie K.

Der Kiemenwulst wird bei Keimen vom Gl.-Stad. 22/23 ein- oder beidseitig exstirpiert. (Abb. 26.) Die Wunde schliesst sich rasch. Körperpigment und Balancer erscheinen normal. Je nach der Grösse des Exstirpationsbereiches fehlen die Kiemen ganz, sind sie rudimentär oder in geringerer Anzahl als normal vorhanden. Von dem Augenblick an, wo die Blutzirkulation einsetzt, muss man zwei Gruppen unterscheiden:

1) Tiere, bei denen im Auge keine Blutzirkulation nachzuweisen ist. Hier treten die gleichen Ausfallserscheinungen auf, wie bei den anderen Versuchsserien, wo Blutzirkulation fehlt (Verspätete Bildung der Augenpigmente, Mikrophthalmie etc.). Die Augengrösse beträgt am Schlusse 65,0 %. Das ist verhältnismässig viel und ist offenbar dadurch zu erklären, dass bei einigen Individuen dieser Gruppe wenigstens zeitweise eine geringe Blutzirkulation stattgefunden hat.

2) Tiere, deren Blutzirkulation im Kopf doch noch normal erscheint. Offenbar haben sich bei ihnen in der Kiemenregion Anastomosen gebildet, durch die der Kopf der Larve trotz der Kiemenexstirpation mit Blut versorgt wird.

Bei dieser Gruppe verläuft das Augenwachstum fast normal. Am Schluss beträgt die Augengrösse 91,9 %. Die Abweichung von der Normalgrösse ist

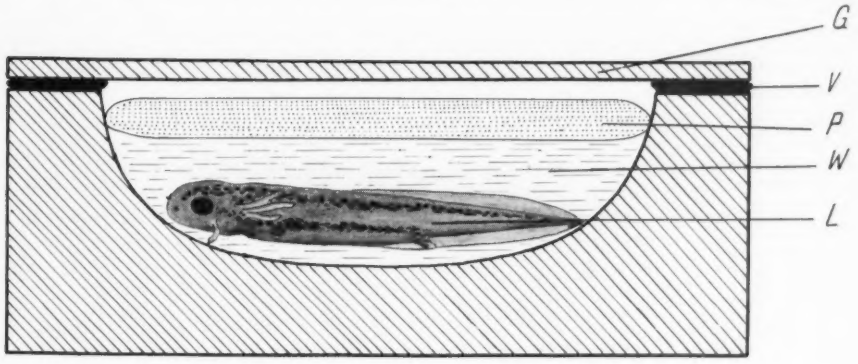


Abb. 27. Larve in sauerstoffarmen Milieu. *G* Glasdeckel, *V* Vaseline-Abdichtung, *P* Schicht von Paraffinöl, *W* Wasser, *L* Larve.

somit gering, aber statistisch gesichert. Trotz einer durch die Operation sicher herabgesetzten Blutversorgung, was die Fälle der ersten Gruppe beweisen und trotz der herabgesetzten äusseren Atmung durch die Verkleinerung der respiratorischen Oberfläche der Kiemen verläuft das Wachstum der Augen fast normal. Dafür gibt es zwei Erklärungsmöglichkeiten:

a) Es ist denkbar, dass das Hautkapillarnetz für die notwendige Sauerstoffversorgung ausreicht, sodass die durch das Fehlen von Kiemen bedingte Verkleinerung der respiratorischen Oberfläche nicht ins Gewicht fällt.

b) Es wäre möglich, dass die primäre Ursache für das verzögerte Augenwachstum nicht in der mangelnden Sauerstoff-Versorgung der Zellen liegt.

In Fällen, wo die Kiemenanlage nur einseitig exstirpiert wurde, lässt sich kein Unterschied in der Augengrösse zwischen dem linken und dem rechten Auge nachweisen.

Serie L.

Triton alpestris-Larven werden vom Gl.-Stad. 33/34 längere Zeit unter Luftabschluss gehalten. 2 ccm ausgekochtes Brunnenwasser, das wieder auf 18° abgekühlt wurde, wurde in einen Glasblock gegossen, die Larve hineingebracht, und das Wasser mit Paraffinöl überschichtet. Das Ganze wurde mit einem Glasdeckel bedeckt, der mit Paraffin abgedichtet wurde. (Abb. 27.) Dadurch wurde die Luftzufuhr von aussen praktisch ausgeschlossen, und die Larve hat zum Leben nur den Sauerstoff zur Verfügung, der sich am Anfang im ausgekochten und abgekühlten Brunnenwasser befand.

Einige Larven wurden in dieser Weise 3, 5, ja sogar 11 Tage weitergezüchtet. Die Entwicklungsgeschwindigkeit ist verzögert; die Kiemen der Tiere werden abnorm gross, was schon DRASTICH (1925) an Salamanderlarven festgestellt hat, aber es kommt in keinem Fall zur Mikrophthalmie.

Zusammenfassend lässt sich sagen, dass Hemmung der äusseren Atmung nicht zur Mikrophthalmie führt. In der folgenden Versuchsserie wurde nun auch die Zellatmung gehemmt.

Serie Q.

Larven von Gl.-Stad. 30 wurden in eine sehr schwache Lösung von KCN gebracht und während einiger Zeit darin aufgezogen. Je 10 Larven wurden in eine Lösung gleicher Konzentration gebracht und in ihrer Entwicklung verfolgt bis zum Tode, oder bis die Kontrollen das Gl.-Stad. 42 erreicht hatten, was 12 Tage dauerte.

Die gesamte Entwicklung bleibt hinter derjenigen der Kontrollen zurück. Diese Hemmung ist aber generell und nicht spezifisch, betrifft also sowohl Kopforgane, wie Extremitäten und Schwanz. Die Wirkung der leichten KCN-Konzentrationen weist äusserlich gewisse Ähnlichkeiten mit der Entwicklung bei niederer Temperatur auf, d. h. es dauert länger bis die Larven ein gewisses Stadium erreicht haben als bei den Kontrolltieren, ohne dass es zu Missbildungen kommt. Die in Tab. 7 zusammengestellten Versuche zeigen, dass die Entwicklungsverzögerung umso stärker ist, je stärker die Konzentration von KCN ist. Trotz diesen Erscheinungen kam es in keinem Fall zu Mikrophthalmie. Wenn die Larven nach einem 5-tägigen Aufenthalt in KCN-Lösung in Brunnenwasser übertragen werden, entwickeln sie sich zu normalen Tieren mit normaler Entwicklungsgeschwindigkeit.

TABELLE 7. Entwicklung der Larven in KCN-Lösungen verschiedener Konzentrationen.

Konzentration von KCN. . .	Erreichtes Stadium beim Absterben, resp. im Zeitpunkt, da die Kontrollen das Gl.-Stad. 42 erreicht haben.
1 %	Höchstens Stadium 31 erreicht; nach 2 Tagen alle tot.
0,1 %	Tiere sterben in den Stadien 31—37 nach 3—10 Tagen Expositionszeit ab.
0,01 %	3 Tiere nach 9 Tagen im Gl.-Stad. 37 abgestorben, die andern erreichen das Gl.-Stad. 38.
0,001 %	Alle Tiere erreichen Entwicklungsstadien von Gl.-Stad. 40—41 nach 12 Tagen.
Kontrollen in Brunnenwasser	Nach 12 Tagen haben alle das Gl.-Stad. 42 erreicht.

b) *Herabsetzung der Ernährung.* (Serie N.)

Nachdem die Versuche mit herabgesetzter Sauerstoffversorgung für die Entstehung der Mikrophthalmie keine Erklärung gaben, wurde in einer weiteren Versuchsserie die Ernährung der Augenzellen vermindert. Während der ganzen Beobachtungszeit ernähren sich die Larven von ihrem Dottervorrat. Für die Augen kommt zuerst der zelleigene Dotter in Frage, später die entodermalen Dotterstoffe, die durch das Blut den Zellen zugeführt werden.

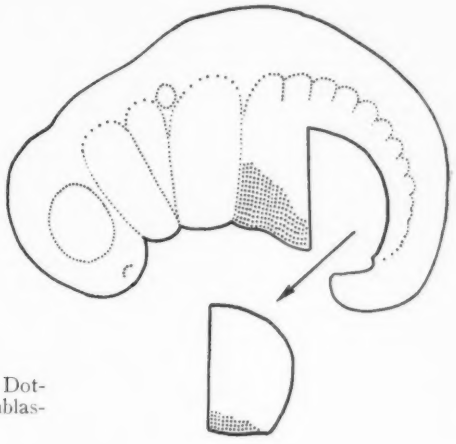


Abb. 28. Exstirpation von entodermalem Dotter. Punktiert: Bereich des Erythrocytenblastems. (Nach SLONIMSKY.)

OL.
31
950

Aus 28 Keimen der Stadien 22/23 bis 25 wird ein Teil des entodermalen Dotters durch Wegschneiden oder Wegpipettieren entfernt. Es ist nicht angebracht, den ganzen Dotterbereich zu exstirpieren, da nach SLONIMSKY (1931) des Erythrocytenblastem, das hinter dem Herz liegt, zum Teil mit der Dotterregion zusammenfällt. Es war daher nur möglich, den hinteren Teil des Dotterbereichs zu exstirpieren. (Abb. 28.)

Die Augen bleiben an Grösse hinter den Augen der Kontrollen zurück. Die Mikrophthalmie ist aber nicht sehr ausgeprägt, ausser in den Fällen, wo keine Blutzirkulation festzustellen ist, weil bei der Operation das Erythrocytenblastem doch verletzt worden ist. Scheiden wir diese Fälle aus und fassen sie zusammen, so zeigt sich, dass bei ihnen das Augenwachstum in gleichem Ausmass verzögert wird wie bei Serie D (Augengrösse im Gl.-Stad. 42 der Kontrollen: 61,2 %). Bei denjenigen Tieren, bei denen die Blutzirkulation gut nachweisbar ist und völlig normal erscheint, beträgt die Augengrösse 83,6 % des Normalen.

Ein Vergleich mit Serie K zeigt, dass bei Dotterexstirpation die Wachstumshemmung fast doppelt so stark ist als bei Kiemenexstirpation, obwohl im ersten Fall die Blutgefässe zwischen Herz und Kopf völlig intakt blieben.

Man kann einwenden, dass auch bei der zweiten Gruppe von Tieren (Augengrösse 83,6 %) das Erythrocytenblastem geschädigt worden sei, wenn auch in geringerem Masse. Das würde bedeuten, dass die dadurch verringerte Menge von Blut die Ursache für das Auftreten des Mikrophthalmus wäre.

Nach den Ergebnissen von Serie H ist anzunehmen, dass die Augengrösse eine direkte Funktion der Blutzirkulation ist. Nimmt man den obigen Einwand als stichhaltig an, wäre zu erwarten, dass die Zwischenwerte der Augengrösse bei den nachweislich nicht durchbluteten Larven und den durchbluteten Larven häufig sind. Die Häufigkeitsverteilung auf die verschiedenen Augen-

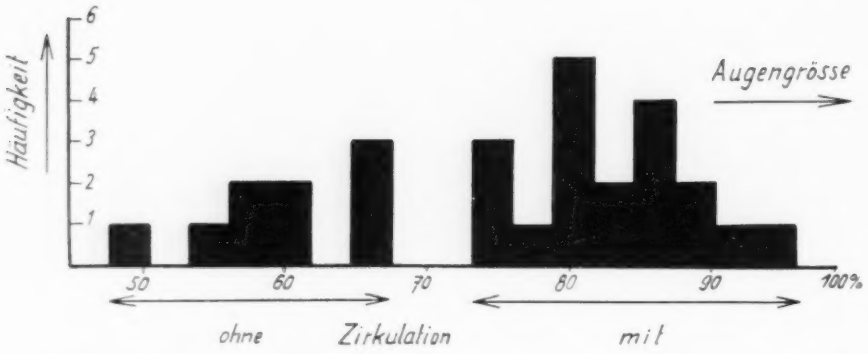


Abb. 29. Häufigkeitsverteilung der Augengrösse bei Larven von Serie X (Exstirpation von entodermalem Dotter). Normale Augengrösse im Gl.-Stad. 42 = 100 %.

grössen (Abb. 29) zeigt aber, dass gerade die ziemlich häufig zu erwartenden mittleren Werte fehlen, sodass sich in der graphischen Darstellung ein Hiat bildet. Der Unterschied der Mittelwerte der beiden Gruppen ist statistisch gesichert ($P < 0{,}001$).

Die Abweichung der Augengrösse vom Normalen beträgt bei den Larven dieser Serie, die Blutzirkulation aufweisen, 13,4 %, also knapp die Hälfte wie bei fehlender Blutzirkulation. Wenn man nun bedenkt, dass bei diesen Larven höchstens die Hälfte des Dotters weggeschnitten werden könnte, so wäre unter der Annahme, dass mangelnde Ernährung der Augengewebe bei der Entstehung der Mikrophthalmie eine entscheidende Rolle spielt, dieses Ausmass der Anomalie zu erwarten.

Demnach ist anzunehmen, dass die nutritive Störung ein wichtiger Faktor für die Entstehung der Mikrophthalmie ist.

V. DISKUSSION.

Bei früheren Autoren, die das normale Organwachstum an *Triton* untersucht haben, wie L. GLAESNER (1925), F. C. E. KNIGHT (1938) und anderen, stand die morphologische Beschreibung zur Aufstellung einer Normentafel der Entwicklung im Vordergrund. In der vorliegenden Arbeit wurde das Hauptgewicht nicht auf die morphologische und histologische, sondern auf die metrische Untersuchung gelegt. In verschiedenen Stadien der Entwicklung wurden mehrere Kopforgane gemessen, ihr Grössenwachstum verfolgt und ihre Wachstumsraten miteinander verglichen. Diese Untersuchung hat ergeben, dass das Organwachstum zuerst mässig schnell verläuft, sich vom Gl.-Stad. 33 an beschleunigt, um sich nach dem Gl.-Stad. 39 wieder zu verzögern. Die graphische Darstellung eines solchen Wachstums ist eine S-förmige Kurve, die

SIMPSON and ROE (1939) als ideale Wachstumskurve beschrieben haben. Die Wachstumsgeschwindigkeiten der verschiedenen Organe zeigen merkliche Unterschiede, wobei aber Korrelationen auftreten; so zeigen Linse, Retina und Zwischenhirn den gleichen Wachstumsrythmus. Dieser Befund stimmt gut mit R. G. HARRISONS (1929) Untersuchungen über die Proportionalität von Augenbecher und Linse überein. Der abweichende Wachstumsrhythmus des Augendurchmessers ist durch eine Besonderheit des Retinawachstums bedingt. Die Dicke der Retina nimmt vom Gl.-Stad. 37 an bei gleichzeitiger Volumenzunahme ab; das Retinagewebe muss sich in dieser Entwicklungsphase flächig ausbreiten.

In der Einleitung wurden zwei Arten von Mikrophthalmie unterschieden, eine früh entstehende, durch Organisatordefekt bedingte, und eine Mikrophthalmie, deren Ursache spätere Wachstumsstörungen sind. Es stellt sich daher die Frage, ob in den Experimenten mit isolierten Kopfstücken nicht doch ein Organisatordefekt für das Auftreten der Mikrophthalmie verantwortlich gemacht werden kann. Das Organisatormaterial invaginiert zwar während der Gastrulation sehr frühzeitig. Immerhin wäre eine spätere Wanderung desselben in cranialer Richtung denkbar. Bei isolierten Kopfstücken müsste eine solche Wanderung unmöglich sein. Die Experimente (Serie D) zeigen jedoch, dass die Hemmung des Augenwachstums gleich stark ist, wenn nur die Herzanlage exstirpiert wird, also nur ein ventraler Defekt gesetzt wird. Eine allfällige späte Kopforganisatorwanderung könnte hier ungestört vor sich gehen. Umgekehrt zeigen HADORNS (1945) Experimente, dass das Setzen eines dorsalen Defektes die Entwicklung der Augen nicht zu stören braucht. Bei der in der vorliegenden Arbeit beschriebenen Mikrophthalmie kann kein Kopforganisatordefekt als Ursache in Frage kommen, wie dies bei den Experimenten von STOCKARD (1913) und ADELMANN (1920), die an früheren Stadien operierten, der Fall war.

Der Grössen- und Formunterschied tritt bei dieser Mikrophthalmie erst in späteren Stadien auf, namentlich erst dann, wenn in den Kontrollen die Blutzirkulation eingesetzt hat. Die abnormale Entwicklung beschränkt sich nicht auf das Auge, sondern es werden auch andere Organe davon erfasst. Wie BAGG and LITTLE (1924) und TWITTY (1932) zeigen, ist dies auch in vielen Fällen erblicher Mikrophthalmie der Fall. Das Fehlen der Blutzirkulation wirkt sich auf das Wachstum der verschiedenen Organe verschieden stark aus; bei den einen Organen wird es früher oder später ganz eingestellt, bei andern nur verzögert. Die Wachstumsgeschwindigkeit ist für die verschiedenen Organe bei normalen Tieren verschieden; aber es lässt sich kein Zusammenhang zwischen der Wachstumsgeschwindigkeit eines Organs bei einem normalen Tier und der Art der Wachstumshemmung bei Zirkulationsausfall feststellen (S. 213).

Um diese spätbedingte Mikrophthalmie zu erzeugen, genügt es, die Herzanlagen zu exstirpieren und dadurch die Blutzirkulation zu unterbinden.

Mehrere Autoren haben Experimente an verschiedenen Objekten ausgeführt, die die Blutzirkulation verunmöglichten. Allerdings stand dabei meist die Frage nach der Entstehung der Blutgefässe im Vordergrund. So hat CHAPMANN (1918) Hühnerembryonen das Herz exstirpiert, hat aber die Augenentwicklung nicht näher beschrieben. LOEB (1893) hat mit chemischen Mitteln bei *Fundulus* die Herzpulsation gelähmt, beschreibt aber nur Embryonen ohne auffallende Missbildungen. KNOWER (1907) hat an *Rana* die Herzanlage operativ entfernt und erhielt Larven mit Missbildungen, z. B. kleinere Augen, abnormales Vorderhirn u. s. w. FREDERICI (1926) und GOSS (1928/29) entfernten bei Amphibien die Bildungszone der roten Blutkörperchen; die Augenentwicklung wird von ihnen aber nicht beschrieben. REAGAN (1917) fand, dass bei Cardiectomie die vorderen Partien stärkere Anomalien aufweisen, während die hinteren Teile normal sein können. Dieser Befund deckt sich mit den Ergebnissen von Serie D. Interessant sind in diesem Zusammenhang Experimente von BAGG und LITTLE (1924). Die Nachkommen von Mäusen, die mit Röntgenstrahlen bestrahlt wurden, wurden mikrophthalm. Zugleich liessen sich häufig Blutungen in der Augengegend nachweisen, was auf schlechtere Durchblutung der Augengegend schliessen lässt. UHLENHUTH (1915) transplantierte Augen bei *Salamandra maculosa*. Er stellte Degeneration, nachher Regenaration auch ohne Licht und ohne Verbindung der Augen mit dem Zentral-Nervensystem fest. Unterschiede in der Regenerationsdauer schreibt er der verschiedenen Zeitdauer der Durchblutung zu. Damit weist er ebenfalls auf die Abhängigkeit der Wachstumsgeschwindigkeit von der Durchblutung eines Organs hin. TRAMPUSCH (1946) hat durch Röntgenbestrahlung von *Amblystoma*-Embryonen mikrophthalme Larven erzeugt. Er führt diese Erscheinung auf Pyknosen zurück, deren Häufigkeit in der cranialen Region der Larve grösser ist als in der caudalen. Daneben erwähnt er aber, dass die Blutzirkulation bei vielen seiner bestrahlten Tiere reduziert war oder fehlte. Wahrscheinlich ist diese Mikrophthalmie dadurch mitbedingt.

Da das Blut Träger von verschiedenen Funktionen ist, stellt sich die Frage, welche Funktion bei den mikrophthalmen Tieren in entscheidendem Masse ausfällt. Dabei kommen vor allem drei Funktionen in Betracht:

1) Die Zufuhr von Sauerstoff zu den Organen.

2) Die Ernährung der Zellen mit zellfremden (entodermalen) Dottersubstanzen in späteren Stadien.

3) Wachstumssteuernde Wirkstoffe, die durch das Blut transportiert werden.

Wie stellen sich die vorliegenden Experimente zu diesen drei Möglichkeiten?

W. M. COPENHAVER und S. R. DETWILER (1948) behandelten Keime vom Blastula-, Gastrula- oder Schwanzknospenstadium an mit stärkeren Konzentrationen von Sulfadiazin und Sulfanilamid. Früh behandelte Embryonen wiesen doppelte Herzen, teilweise Cyclopie, persistierenden Dotterpfropf oder andere Missbildungen auf. In späteren Stadien behandelte Embryonen wurden anämisch, wobei sich ihre Entwicklung verzögerte. Der Einfluss dieser Behandlung auf die Augenentwicklung wurde von den beiden Autoren nicht näher beschrieben.

VOL.
31
195

1) Sauerstoffzufuhr.

Trotz der bei der Kiemenexstirpation sicher beeinträchtigten Blutzufuhr zu den Kopforganen kommt bei der Messung des Augendurchmessers nur ein Ausfall von ca. 8 % zustande. Es besteht zwar eine ganz schwache Korrelation zwischen der Anzahl der an der Larve verbliebenen Kiemen und der Augengrösse, was aber durch verschieden gute Blutversorgung der Kopforgane zu erklären ist.

Bei Larven, die längere Zeit (Bis 11 Tage) unter Luftabschluss gehalten wurden, werden die Kiemen abnormal gross, was auch von DRASTICH (1925) gefunden wurde, ohne dass die Augengrösse relativ zurückbleibt.

Das Halten von Larven in KCN-Lösung ist ohne Einfluss auf die relative Grösse der Augen. Es kam zu einer generellen Hemmung des Wachstums, das sich nachher in normalem Milieu ausgleicht, ohne dass in irgend einem Stadium Mikrophthalmie festzustellen ist.

Alle diese Tatsachen machen es unwahrscheinlich, dass mangelnde Sauerstoffzufuhr durch das Blut die entscheidende Ursache dieser Mikrophthalmie ist.

2) Nutritive Wirkung.

In diesem Zusammenhang sind Arbeiten von HARRISON (1929), TWITTY und ELLIOT (1934) und MOMENT (1933) von besonderem Interesse. Diese Autoren zeigen, dass das Augenwachstum grossenteils durch inhärente Faktoren gesteuert wird. Nach MOMENT (1933) können die Augen auch wachsen, wenn das Wachstum des übrigen Körpers still steht. HARRISON (1929), sowie TWITTY and ELLIOT (1934) haben Augen heteroplastisch zwischen *Amblystoma tigrinum* und *A. punctatum* transplantiert. Die transplantierten Augen von *A. tigrinum* wachsen sogar schneller als bei den Kontrollen. TWITTY and ELLIOT schreiben diese Erscheinung der verschieden guten Ernährung der Augengewebe zu, die vom Wirtstier abhängig ist; denn diese Unterschiede verschwinden bei maximaler Fütterung aller Tiere.

Während der ganzen Beobachtungszeit ernähren sich die Larven in meinen Experimenten von ihrem Dottervorrat, wobei zuerst der zelleigene, später der entodermale gebraucht wird. Im Zeitpunkt der ersten Nahrungsaufnahme von aussen mussten die Experimente abgebrochen werden, da die Kopfstücke keine Nahrung aufnehmen konnten und damit die ernährungsphysiologischen Bedingungen gegenüber den Kontrollen nicht mehr vergleichbar wären.

Entfernt man operativ die Hälfte des Dottervorrates, so werden die Augen um 16 % kleiner als normal, während sie bei Ausfall der Blutzirkulation um 42 % kleiner werden. Im zweiten Fall müssen die Augengewebe auf den gesamten entodermalen Dottervorrat verzichten, der ihnen infolge der fehlenden Blutzirkulation nicht zugeführt werden kann, während im ersten Fall wenigs-

tens der im Körper verbliebene Teil des entodermalen Dottermaterials zur Ernährung der Augen beitragen kann. Demnach ist die Wachstumshemmung im zweiten Fall 2 bis 3 mal grösser. Die Einwände gegen dieses Experiment wurden schon erwähnt (S. 223).

Es ist daher anzunehmen, dass nutritive Störungen bei der Entstehung dieser Mikrophthalmie von Bedeutung sind.

3) Hormonale Wirkung oder Ernährung.

Über hormonale Wirkung sagen meine Experimente nichts direkt aus. Ich habe durch Einlegen von Larven in Thyroxin-Lösung versucht, den Grundumsatz zu steigern, was aber ohne Einfluss auf die Augengrösse von Tieren blieb, denen vorher das Herzblastem entfernt worden war.

Bei diesem Problem muss ich nochmals auf die Ergebnisse von Serie H („Halbparabiosen") zurückkommen. Nimmt man an, dass das Wachstum in erster Linie durch inhärente Faktoren gesteuert wird und von Seite des Blutes nur eines auslösenden und regulierenden Faktors bedürfe, um normal auszuwachsen, wäre zu erwarten, dass die Augengrösse der Augen bei den „Halbparabiosen" normal wäre. Das ist aber nicht der Fall, sondern das Auge bleibt um 12 %, resp. 20 % kleiner als normal, wobei die Unterschiede gesichert sind. Auf jeden Fall spielt die Ernährung der Augengewebe für ihr Wachstum eine entscheidendere Rolle (S. 224).

VOL. 31 1950

VI. ZUSAMMENFASSUNG.

1) Bei Keimen und Larven von *Triton alpestris* wurde das normale Wachstum der Kopforgane untersucht. Die Wachstumskurven haben meist S-Form. Bei allen Organen verläuft das Wachstum zwischen den Glaesner-Stadien 39 und 42 langsamer als unmittelbar vorher.

2) Die Untersuchung der arithmetischen Wachstumsgeschwindigkeit zeigte, dass Zwischenhirn, Retina und Linse den gleichen Wachstumsrhythmus haben, wogegen das Wachstum von Auge und Kopfbreite anders verläuft.

3) Kopfstücke von *Triton alpestris*-Embryonen, die im Schwanzknospenstadium isoliert werden, weisen Mikrophthalmie auf. In vielen Fällen ist auch die Form der Augen abnormal. Eine extrem abnormale Form, das „Zackenauge", tritt umso häufiger auf, je näher die Schnittstelle bei den Augen liegt.

4) Die Kerne der Retinazellen sind bei den Kopfstücken gleich gross wie bei den Kontrollen. Der Grössenunterschied der Organe ist durch die geringere Zellzahl bedingt. Dementsprechend ist die Mitoserate bei den Kopfstücken niedriger als bei den Kontrollen.

5) Für die Entstehung der „spätbedingten Mikrophthalmie" kann nicht ein Kopforganisatordefekt verantwortlich gemacht werden.

6) Mikrophthalmie trat in meinen Versuchen bei Zirkulationsausfall immer auf. Herzexstirpation führt bei Triton alpestris-Larven zu einem Stillstand des Augenwachstums.

7) Der Zirkulationsausfall wirkt sich auch auf andere Organe aus. Die Wachstumshemmung ist für verschiedene Organe verschieden gross. Die Hemmung der Wachstumsgeschwindigkeit erfolgt nicht proportioniert, sodass Köpfe mit disproportionierten Organen entstehen.

8) Bei Hemmung der Atmung trat in meinen Versuchen keine Mikrophthalmie auf.

9) Partielle Exstirpation von entodermalem Dotter führt zu einer Hemmung des Augenwachstums. Daraus wird geschlossen, dass nutritive Faktoren für die Entstehung der in dieser Arbeit beschriebenen Mikrophthalmie von entscheidender Bedeutung sind.

VII. LITERATURVERZEICHNIS.

Adelmann, H. B. 1930. Experimental study of development of eye. J. of exp. Zool. 57.

Ashley, L. M. 1947. Bilateral anophthalmus in a brother and sister. J. of Heredity 38.

Bagg, H. J. and Little, C. C. 1924. Hereditary structural defects in the descendants of mice exposed to Roentgenray irradiation. Am. J. of Anatomy 33.

Bardeen, Ch. R. 1907. Abnormal development of toad ova fertilized by spermatozoa exposed to the Roentgen rays. J. of exp. Zool. 4.

Bourne, M. C. and Grüneberg, H. 1939. Degeneration of the retina and cataract. J. of Heredity 30.

Brachet, J. 1944. Embryologie chimique. Paris & Liège 1944.

Carris, L. H. 1932. Hereditary blindness. J. of Heredity 23.

Chapman, W. B. 1918. The effect of the heart-beat upon the development of the vascular system in the chick. Am. J. of Anatomy 23.

Chase, H. B. and Chase, E. B. 1941. Studies on an anophthalmic strain of mice. J. of Morphology 68.

Clark, W. E. le gros and Medawar, P. B. 1945. Essays on growth and form, presented to d'Arcy Wentworth Thompson. Oxford 1945.

Copenhaver, W. M. and Detwiler, S. R. 1948. The effects of sulfonamids on Amblystoma embryos, with particular reference to blood development. J. of exp. Zool. 109.

Drastich, L. 1925. Über das Leben der Salamandra-Larven bei hohem und niedrigem Sauerstoff-Partialdruck. Zeitschr. f. vergl. Physiologie 2.

Eaton, O. E. 1937. A hereditary eye defect in guinea pigs. J. of Heredity 28.

Eigenmann, C. H. 1909. Cave vertebrates of America. Carnegie publication of Washington 104.

Fischel, A. 1921. Über normale und abnormale Entwicklung des Auges. W. Roux' Archiv 49.

Franceschetti, A. 1930. Die Vererbung von Augenleiden. Schieck & Brückners Handb. d. Ophthalm. Bd. 1.

Frederici, E. 1926. Recherches experimentales sur les potencialités de l'ilot sanguin chez Rana fusca. Archiv de biologie 36.

Fueter, R. 1930. Das mathematische Werkzeug. Zürich 1930.

GLAESNER, L. 1925. Normentafeln zur Entwicklung des gemeinen Wassermolches. Jena, G. Fischer 1925.

GOSS, CH. 1928. Experimental removal of the blood islands of Amblystoma punctatum embryos. J. of exp. Zool. 52.

GUYER, M. E. and SMITH, E. A. 1918. Some prenatal effects of lens antibody. J. of exp. Zool. 26.

— 1920. Transmission of induced eye defects. J. of exp. Zool. 31.

HADORN, E. 1945. Beitrag zur Entstehung der Mikrophthalmie. Julius Klaus Archiv. 20.

— 1947. Über einen Einfluss des embryonalen und frühlarvalen Gehirns auf die Gestalt der Rumpf-Schwanzregion bei Triton. Revue suisse 54.

HAIN, A. M. 1933. Development anomalies in the Wistar albino rat. Nature 32.

HALE, F. 1933. Pigs born without eyeballs. J. of Heredity 24.

HARRISON, R. G. 1929. Correlation in the development and growth of the eye studied by means of heteroplastic transplantation. W. Roux' Archiv f. Entw. Mech. 120.

HERTWIG, P. 1942. Neue Mutationen und Koppelungsgruppen bei der Hausmaus. Zeitschr. f. ind. Abst. u. Vererbungsl. 80.

HOLLAENDER, W. F. 1948. Microphthalmia and other eye defects in pigeons. J. of Heredity 39.

HUMPHREY, R. R. 1924. A case of cyclopia in Homo. An Record 28.

HUXLEY, J. 1932. Problems of relativ growth. London 1932.

JEFFREY, F. R. 1941. Hereditary microphthalmia in the domestic fowl. J. of Heredity 32.

KING, H. D. 1931. Studies on the inheritance of structural anomalies in the rat. Am. J. of Anatomy 48.

KNIGHT, F. C. E. 1938. Die Entwicklung von Triton alpestris bei verschiedenen Temperaturen. W. Roux' Archiv f. Entw. Mech. 137.

KNOWER, H. Mc. E. 1907. Effects of removal of the heart and arrest of circulation in the development of frog embryos. An. Record 7.

LANDAUER, W. 1933. Untersuchungen über das Krüperhuhn. Zeitschr. f. mikr. anat. Forschung 32.

LEHMANN, F. E. 1945. Einführung in die physiologische Embryologie. Basel 1945.

LINDER, A. 1945. Statistische Methoden. Basel 1945.

LOEB, J. 1893. Ueber Entwicklung von Fischembryonen ohne Kreislauf. Pflügers Archiv 54.

— 1915. The blindness of the cave fauna and artificial production of blind fish embryos by heterogeneous hibridization and by low temperatures. Biol. Bull. Woods Holl 29.

MANGOLD, O. 1931. Das Determinationsproblem III. Das Wirbeltierauge in der Entwicklung und Regeneration. Erg. d. Biol. 7.

MOMENT, G. B. 1933. The effects of rats of growth on the postnatal development of the white rat. J. of exp. Zool. 65.

PICTET, A. et FERRERO, A. 1940. Hérédité de l'anophthalmie et de malformations oculaires dans la descendance d'un croisement interspécifique de Cobayes. Revue suisse 47.

REAGEN, F. P. 1917. Experimental studies on the origin of vascular endothelium and of erythrocytes. Am. J. of Anatomy 21.

ROBERTS, L. D. 1948. Microphthalmia in swine. J. of Heredity 39.

SEEFELDER, R. 1930. Missbildungen des menschlichen Auges. Schieck & Brückners Handb. d. Ophthalm. 1.

SIMPSON, G. G. and ROE, A. 1939. Quantitative zoology. New York and London 1939.

SLONIMSKY, P. 1931. Recherches experimentales sur la génèse du sang chez les Amphibiens. Arch. de biologie 42.

SPEMANN, H. 1936. Experimentelle Beiträge zu einer Theorie der Entwicklung. Jul. Springer, Berlin 1936.

VOL. 31 1950

STOCKARD, CH. R. 1907. The influence of external factors, chemical and physical, on the development of Fundulus heteroclitus. J. of exp. Zool. 4.

— 1909. The development of artificial produced cyclopean fish. J. of exp. Zool. 6.

— 1913. An experimental study of the position of the optic anlage in Amblystoma punctatum with a discussion of certain eye defects. Am. J. of Anatomy 15.

TRAMPUSCH, H. A. L. 1946. Een en ander over de genese van den kop. Ned. Tijdschrift voor Geneskunde 90.

TWITTY, V. C. 1932. Influence of the eye on the growth of its associated structures, studied by means of heteroplastic transplantation. J. of exp. Zool. 61.

TWITTY, V. C. and ELLIOT, H. A. 1934. The relative growth of the Amphibian eye, studied by means of transplantation. J. of exp. Zool. 68.

UHLENHUTH, E. 1915. Are function and functional stimulus factors in producing and preserving morphological structure? Biol. Bull. Woods Holl 29.

VOL.
31
1950

ON THE PRONEPHROS AND THE BLOOD IN MYXINE GLUTINOSA

BY

NILS HOLMGREN

CONTENTS.

Introduction 234
General morphology of the pronephros 238
External appearance of the pronephros 238
The general structure of the pronephros 239
The general appearance of the central duct 241
The blood supply of the pronephros 243
The general structure of the central mass 244
The pronephric tubules 246
The formation of new tubules 249
The fibrous tissue of the central mass 252
The collagenous structures of the head kidney 255
The contents of the blood sacs 260
Epitheloid alveoles in the central mass 262
The distribution of the fat in the pronephros 263
The glomus 264
The excretory cells 265
The collagenous structures of the glomus 266
The capillary convolutions of the glomus and their contents 266
Degeneration and regeneration of parts of the glomus 270
Mesonephric glomeruli 271
Formation of the blood corpuscles 272
Formation of red blood corpuscles: 272
a) in the spleen 272
b) in the liver 274
c) in the pronephros 274
Formation of red blood corpuscles 274
Formation of white blood corpuscles 277
Phagocytes 278
The spindle cells 281
The megacaryocytes 283
The blood of Myxine as it appears in blood smears 286
Erythrocytes 286
Haemocytoblasts 288
Proerythroblasts 288
Polychromatic erythroblasts 289
Normoblasts 290
Leucocytes or granulocytes 290
Monocytes 291
Lymphocytes 291
Thrombocytes 291
Spindle cells 291
Phagocytes 292

Heparoblasts 292
Megacaryocytes 292
Microcytes 292
Cells degenerating in the circulating blood 293
Erythrocytes 293
Haemocytoblasts, proerythroblasts and polychromatic erythroblasts . . . 294
Lymphocytes and spindle cells 295
Granulocytes 296
Other blood elements 296
The pronephroi of specimens of different sizes 296
The morphological aspects of the functional development of the head kidney . 340
Periodical activity of the head kidney 343
Short summary 345
Literature cited 347

INTRODUCTION.

The first description of the pronephros of Myxinoid fishes seems to be that of JOHANNES MÜLLER (1845). He described it as a peculiar grapelike gland and considered it to be a suprarenal organ. A. RETZIUS, however, presumed that it is a kidney. Both authors stated that this organ has no outlet. The first detailed description of the pronephros, however, was given by WILHELM MÜLLER in 1875. According to him the pronephros of *Myxine* is connected with the segmental (mesonephric) duct through a slender canal, which frontally widens to an oblong body (the pronephros), the dorsal and lateral surface of which is covered by short tubuli opening into the pericardial cavity. The ventral wall of the pronephros has, according to him, a small number of "Ausbuchtungen", each containing a glomerulus. The tubuli consist, according to W. MÜLLER, of cylindrical epithelial cells, completely devoid of cilia.

WELDON (1884) studied the pronephros of *Bdellostoma forsteri*, var. *hexatrema*. In this species no connection exists between the pronephros and the segmental duct. The non-ciliated epithelium of the tubules consists of columnar cells, outside which a basal membrane is developed. Outside this a small quantity of connective tissue and an exceedingly rich capillary plexus is said to be present. Thus a considerable quantity of blood passes between the tubules. The latter unite basally with one another and finally open into the *central duct*. This duct contained a blood-clot. Caudally it formed a "lymphatic" body connected with a glomerulus. The pronephros of the right side is connected with the adventitia of the portal vein, the left with the anterior cardinal. WELDON considers the pronephros to be a suprarenal organ.

GROSGLIK (1885) expressed the opinion that the pronephroi described by W. MÜLLER and WELDON belonged to immature animals, with not yet reduced but reducing pronephroi. His assumption was based upon theoretical interpretations.

VOL. 31 1950

According to KIRKALDY (1894) the pronephros of *Myxine* is totally cut off from the segmental duct. It has a very intimate connection with the "postcardinal" vein and a great part of it is lodged in the vein. A glomerulus is present at the caudal end of the pronephros. A central duct is present. In a specimen without eggs this duct is said to give off outgrowths containing glomeruli, with blood-vessels forming characteristic capillary loops. These blood-vessels are depicted as uniting the different glomeruli with one another. The central duct is covered with a colomnar epithelium with a well-defined basement membrane. The columnar cells of the tubules show distinct traces of a ciliation. In another specimen, containing large eggs, the pronephros has a different appearance. The inner part of the posterior tubes is much altered. Their lumen is diminished or obliterated and the columnar cells are no longer recognizable. Some of these cells have become fibrous with small nuclei, others have been enlarged, rounded, with a greatly swollen nucleus. No central duct or glomerulus can be made out. The space where the central duct should be is occupied by the central mass, a reticulum of protoplasm containing small cells with small nuclei and larger cells with very large, round nuclei. Blood-vessels are said to enter this tissue and break up into fine capillaries. The central mass appears to be derived from the central duct of earlier stages. According to KIRKALDY the two pronephroi described are different stages in the development of this organ. "The change consists in the formation of a mass of tissue much resembling lymphatic tissue, which would appear to result from the breaking down of the walls of the ducts, the constituent cells furnishing the fibrous groundwork of the new tissue, and also cells with large nuclei, which I am inclined to regard as formative cells, since in the most reduced organs I have examined they have disappeared, their place being taken by many smaller cells." KIRKALDY seems to consider the pronephros to represent a suprarenal organ.

Between 1896 (and 1897) and 1910 a number of papers on the pronephros in Myxinoids appeared, viz. the papers of PRICE (1896, 1897, 1904, 1910) on the early development of the pronephros of *Bdellostoma stouti*, SEMON's (1896, 1897) and SPENGEL's (1897, a, b) animated battle on the pronephros of *Myxine*, and MAAS' (1897) paper on the kidney system in *Myxine*.

From PRICE's papers of 1896, 1897 and 1904 I shall here quote only a few points. 1) He found that the rudiment of the pronephros is composed of segmentally arranged tubuli connected with a segmental duct derived from the tubuli (nephrotomes). Caudally it continues with the "mesonephric duct." 2) The segmental pronephros becomes shortened through the crowding together of the tubules into one or two segments.

According to SEMON (1896) the pronephros of *Myxine* has no central duct. A number of tubuli frequently unite to form a common duct which opens through one or many openings into an interior cavity or into a system of cavities situated in the interior of a tissue containing blood-vessels. This tissue

is said to consist of a number of glomeruli. SPENGEL (1897, a) denies the presence of these glomeruli and considers the central mass to be composed of metamorphosed epithelial cells of the tubuli. He considers the pronephros of *Myxine* to be probably a supra-renal organ or a lymphoid organ. The polemical papers of SEMON (1897) and SPENGEL (1897, b) offer no very great interest with regard to the morphology of the pronephros and will therefore not be treated here.

MAAS (1897) has published an extensive description of the pronephros in *Myxine*. He had at his disposal a large number of specimens of different sizes. His youngest specimens were those of 8.5, 9.8 and 13 cm body length, his oldest measured 45 cm. In his youngest stage the tubuli are said to be located in two longitudinal rows, a dorsolateral and a medioventral. The central mass forms no continuum but consists of a limited number of totally independent parts. In each portion blood-vessels are said to be present. In older animals the central mass seems to form a continuum throughout the entire pronephros, but in it the original partitions are said to be recognizable. The central mass is derived from the internal end of the tubuli, through transformation of their epithelial cells. The mass consists essentially of cell-strings mostly arranged longitudinally. The cells frequently form nets. Then their nuclei are small and round. There are also giant cells with very large nuclei present. The central mass ("strittige Gewebe", MAAS) becomes more and more "lymphatisch" and is not unlike a supra-renal organ, i.e. its core-substance. The epithelial cells of the tubules have strong cilia. The central mass acquires a capillary system through aortal branches, which are depicted as piercing the wall of the vein sinus into which the central mass swims. These arterial branches are depicted as traversing the lumen of the vein to reach the central mass.

PRICE (1910) examined the head kidney of *Bdellostoma stouti*. He made the statement that while the examined kidneys (more than two dozen) "agree in essential features, there is still much variation in detail, so much that an exact description of one could not answer in detail for any other." He described the ciliated tubuli and the central duct. The latter has openings into the vein sinus even in very young specimens. The central mass is variably developed, consisting of the modified epithelial tissue of the central duct with loosely arranged cells. Between the tubules are sinusoids opening in the vein sinus. No arteries were observed. PRICE considered that the function of the head kidney is to transfer body fluid from the body-cavity to the blood. But the kidney may have some other function or functions. "But, however this may be, it is certain that excretion cannot be one of them, as there is no connection with the exterior."

The most exhaustive description of the pronephros of Myxinoids is that of CONEL (1917). CONEL has treated *Bdellostoma stouti* as well as *Myxine*.

The central duct of *Bdellostoma* consists mostly of a number of independent

VOL. 31 1950

segments, each of which opens into the vein sinus. Sometimes, however, there was a continuous duct present. The cells of the central duct are columnar, usually larger than those of the tubuli. The basal membrane of the cells of the tubuli is continuous with the basal membrane of the duct. The width of the duct is very variable. The duct is partly surrounded by the the "central mass." The mass is more or less cylindrical. "In none of the specimens does it entirely fill the lumen of the vein. The endothelium of the pronephric vein continues on the surface of the mass. "There is no definite cellular structure observable in the central mass." The mass is composed of a large number of cells, with more or less oval nuclei. Large, small and intermediate sizes are mingled irregularly. Some cells are isolated, others form groups of different sizes. There seems to be a granular cytoplasmic groundwork, scattered throughout the central mass. "There is no connective tissue in the central mass." Giant cells occur in small numbers. They are interpreted as enlarged blood corpuscles. They are surrounded by a thin membrane, interpreted as an endothelium. Real blood corpuscles were only rarely seen in the central mass. There are many spaces in the central mass, but they are never lined by endothelium and never contain blood corpuscles. No blood-vessels were seen emerging from the central mass. The central mass is completely shut off from the pronephric vein. The openings of the central duct into the vein are lined by the same endothelial sheath as that which surrounds the mass. In the lumen of the central duct near its openings lies a collection of nuclei and granules, which have the appearance of vast particles. Blood corpuscles are common in the central duct and in the basal part of the tubules. No blood was in the pericardial cavity. No lymphatic tissue is present.

The pronephros of *Myxine* contains much more connective tissue than that of *Bdellostoma*. The tubules are surrounded by much more connective tissue, and such tissue even extends into the vein. In an old specimen the amount of connective tissue is far greater than in younger animals, and the mass contains many blood-vessels. In *Bdellostoma* the inner ends of the tubules are always closed, whereas they are seldom so in *Myxine*. The inner ends of the tubuli of the latter abut on to a loosely arranged mass of cells "bordering on or lying in the pronephric vein." The central duct is much more reduced in *Myxine* than in *Bdellostoma*, seldom with the epithelial wall intact.

The central mass is different from that in *Bdellostoma*. In immature animals its conditions approach those in *Bdellostoma*. It contains more or less oval nuclei in a groundwork consisting of connective tissue. In older animals the central mass has no definitive form and consists of a loose collection of nuclei along the base of the tubuli. In occassional places the vein lumen communicates with the central duct. In the posterior part of the pronephros the mass becomes compact. The central mass is connected with the wall of the vein by broad bands of connective tissue. Blood-vessels run from the connective tissue at the

base of the pronephros into these bands. Giant blood corpuscles are very numerous and are contained in capillaries. "In the posterior region of the pronephros in *Myxine* the central mass resembles a lymphatic structure." Characteristic of the pronephros of *Myxine* are the broad bands of connective tissue which extend through the pronephric vein dividing its lumen more or less completely into two parts. Near the Malpighian body a large, isolated thick-walled duct begins blindly and ends blindly. These structures are interpreted as degenerating Malpighian bodies.

In my paper of 1945 I have described the pronephros of two embryos of *Myxine*. From this description I here quote that there is a *segmental* duct into which the tubuli open. In the older of the two embryos the lumen of this duct is becoming obliterated. The glomus of the pronephros appears as 3 open, rounded chambers developed at the bottom of folds of the pericardial wall at the caudal part of the kidney, without any connection with the tubuli or the segmental duct. The blood circulation in the kidney is effected by the pronephric vein (developed as a vein pocket) connecting with a number of veins proceeding from the yolk sac to the kidney. These veins form a plexus around the tubules and connect between the tubules with the wide pronephric vein pocket.

VOL. 31 1950

GENERAL MORPHOLOGY OF THE PRONEPHROS PROPER.

EXTERNAL APPEARANCE OF THE PRONEPHROS.

CONEL (1917) has described the pronephroi of *Bdellostoma* as well as those of the North-American *Myxine*. There is this difference between these two, that the tubules seem to be shorter in *Bdellostoma* than in *Myxine*, so that they do not protrude over the surface of the pronephros, as they generally do in the latter. The variability, however, is very wide, so wide in fact that no general description could be made beyond the fact that the pronephros is an elongated body more or less distinctly lobated with tubules opening into the pericardial cavity.

The size of the pronephros is also very variable. In the North-American *Myxine*, which is very much larger than that of Gullmaren Fjord, CONEL found the pronephros of seven specimens with a body length of 58—68 cm, varying between 5.5 and 10.25 mm. The *Myxine* of Gullmaren Fjord seldom seems to be larger than 40—46 cm, and the biggest pronephros met with had a length of 6 mm. This was in an animal of 38 cm body length. CONEL has found that "there is a correlation between the size of the pronephros and the length of the animal in *Myxine*, but not a sufficiently large number of specimens was available to prove this to be constant." "The right pronephros of *Myxine* was almost always larger than the left."

In the Gullmaren specimens the right pronephros is generally larger than the left, but there are many exceptions, the two pronephroi sometimes being about the same size or the left considerably larger than the right. The two pronephroi, moreover, are mostly differently shaped, the right being compressed, protruding into the pericardial cavity, triangular, the left flat and not noticeably protruding. Between these two forms there are many transitions, which will not be described here.

A correlation between the size of the animals and their pronephroi undoubtedly exists, but this correlation is quite average, so that the largest animal has pronephroi bigger than the small or medium-sized animals. But a large animal may have small, and a somewhat smaller one large pronephroi. For instance, the 38-cm animal mentioned above has two pronephroi, each measuring 6 mm in length, whereas another animal of the same size has pronephroi measuring 3 mm. The same phenomenon seems to occur in all size classes. In each there are animals with small and animals with large, and between them animals with medium-sized, pronephroi. The irregularity in the sizes of the pronephroi might be due to four causes: 1) Grade of ontogenetical development, 2) a variability, 3) different functional stages or 4) all three together. In addition, there are differences in size due to degeneration of the kidney.

These possibilities cannot be fully considered before the histology of the pronephros has been treated. Here I shall only observe that a variability which does not depend upon functional stage or upon ontogenetical development occurs. This is clear from the following facts. There are in animals of every size some with large pronephroi. Such pronephroi always have a very large number of closely set tubuli. In the same class of animals there exist those which have small pronephroi with a relatively small number of tubuli. The latter type of pronephros could easily be transformed into the former by growing more tubuli, but the former with its numerous tubuli could scarcely transform into a type with few tubuli. This would involve a very extensive degeneration and reduction of tubuli, which undoubtedly must presuppose a common occurrence of degenerating tubuli in the sections. In reality such tubuli do occur, though rather exceptionally, as will be demonstrated below. I must therefore assume that there is great variability in the size and form of the tubuli present, a variability that causes many difficulties when we try to explain the functional development of the pronephroi.

THE GENERAL STRUCTURE OF THE PRONEPHROS.

In a very young embryo (of *Bdellostoma*) the rudiment of the pronephros consists of a number of segmentally arranged tubuli, apically opening into the pericardial cavity and basally joining the *segmental* duct which caudally continues into the mesonephric duct. Later the segmental arrangement is

erased, as the tubules are crowded together, mostly into two segments. At the same time the segmental duct seems to lose its lumen, its cells crowding together as is the case in the older of my *Myxine* embryos, which I described in my paper of 1945. Pronephric glomeruli are forming. In this embryo the pronephric vein has made connection with the pronephros, and a pronephric circulatory system has arisen. Later we find the basal parts of the pronephros immersed into the pronephric vein. This basal part is the central mass, which is thus floating into the blood stream of the pronephric vein (dorsal part of the anterior cardinal vein, COLE (1914). Now the essentials of the future pronephros are established. Although these essentials are well known, I shall here give a summary description.

The pronephros consists of three distinct parts: the tubuli, the central mass and the glomus. (Fig. 1.)

The tubuli open apically into the pericardial cavity and are basally connected with the central mass. The latter is an elongated cell mass. It has a very variable appearance. Sometimes it forms a narrow string, sometimes a bulky mass, and between these two extremes it may have any appearance. The tubuli are connected with the central mass in different manners. Sometimes the tubuli basally form a small closed vesicle which dips into the cell mass of the central mass; sometimes this vesicle lacks a proper basal wall and then may be described as a reversed funnel set into the central mass. However it may be formed, one thing is certain, viz. that there is a cavity at the basal end of the tubules. This cavity is the *central cavity* (of this paper). Adjacent central cavities are seldom joined on to one another to form a "duct"—the *central duct* of the authors. This "duct" (in *Myxine*) is generally divided into sections, shut off from each other. The "central duct" must not be confused with the "segmental duct," which has disappeared before the "central duct" arises. Very often vacuities are present or arise outside the central cavity. Such vacuities are here termed "central vacuities." They arise mostly through the coalescence of smaller hollows in the central mass or through the destruction of central parts of this mass. The vacuities are never lined with epithelial or endothelial cells. Sometimes they open into the pronephric vein.

The pericardial openings of the tubuli are surrounded by the pericardial epithelium. This epithelium at first follows the wall of the tubule somewhat basalwards, then bends over to the neighbouring tubule, to follow it to its apex. Thus there originate between the tubuli (triangular) spaces, formed by the pericardial membrane and the tubulus. These spaces are occupied by a single blood sinus, which is continuous with the pronephric vein lumen. These blood spaces are clad with an endothelium, continuous with that of the pronephric vein. The endothelium of the pronephric vein envelopes the tubules basally but does not extend from there to form a lining membrane upon the central mass.

VOL. 31 1950

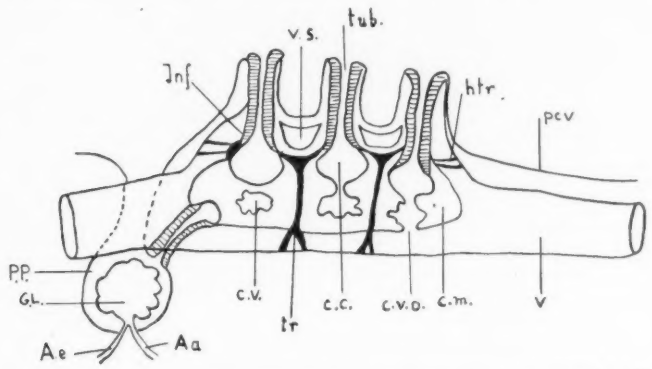


Fig. 1. Pronephros of Myxine. Diagrammatical representation. Aa, arteria afferens; A.e., Arteria efferens; c.c., central cavity; c.m., central mass; c.v., central vacuity; c.v.o., opening of central vacuity in the pronephric vein; G.L., glomus; htr., horizontal trabecle, Inf, Infundibulum; p.c.v., pericardial wall; P.P., pericardial pocket; tr., trabecle; tub., tubule; v, pronephric vein; v.s., vein sinus.

The pronephros may thus be described as an organ suspended at the pericardial wall and with its central mass immersed into the pronephric vein.

Even in young specimens the bases of the tubules are surrounded by a collagenous ring consisting of a thickened portion of the collagenous basement membrane of the basal columnar cells together with the collagen connective tissue of the pronephric vein. In older specimens this ring is very thick, built up of coarse collagenous bundles partly immersed into the venous spaces lying between the tubules. Through these bundles the venous spaces around the basal parts of the tubules are divided up to form a strong venous plexus.

In this general description of the pronephros of *Myxine* the central mass is interpreted as a single cell mass extending throughout the entire kidney. This statement, based upon hundreds of sectional series, disputes the statements of Maas (1897), according to which the central mass in his youngest specimen (8.5 cm body length) consists of as many independent segments as there are groups of tubuli. In spite of my having no 8.5 cm specimen at my disposal, I must declare that Maas's statement must be incorrect as in the 4.3 and 9.6 cm specimens which I have investigated there is no such subdivision of the central mass. His statement must be due to an insufficient combination of the sections of the series. Other misinterpretations and obvious errors in his description bearing upon this question will be mentioned later in connection with the question of the blood supply of the central mass.

THE GENERAL APPEARANCE OF THE CENTRAL DUCT.

In *Bdellostoma* there may sometimes be a central duct continuous throughout the entire pronephros (Price 1910, Conel 1917), but usually the duct consists

of a series of cavities, each belonging to a smaller or larger group of tubuli opening into it. This duct is said to have openings to the pronephric vein (through the central mass), so that the vein is in open communication through the tubuli with the pericardial cavity.

In *Myxine* there is never a continuous central duct throughout the entire pronephros. "The central duct" is always divided in a number of compartments each with its supply of tubuli. (Fig. 2.) These divisions may be of very different length, connected with a very different number of tubuli. There seems to be a certain correlation between the length of the compartments and the length or age of the animal. This correlation seems to depend upon the mode of growth and branching of the tubular system, as will be discussed further on in this paper.

The simplest, but not necessarily therefore the primitive form of a central duct or *cavity*, is when in certain cases it consists of the somewhat widened base of a basally closed tubule dipping into the central mass. (Fig. 2.) The cells closing this tubule are more or less modified columnar cells, provided with their ciliar apparatus. These cells are most modified in the internal wall of the cavity. In this simple case the central cavity is quite empty, not containing cells of any description. Sometimes the basal part of the tubulus is not widened, dipping into the central mass.

In most cases, in older animals, the central cavity may be described as being formed of the basal part of a tubule (branched or unbranched) that is sunk like an inverted funnel into the central mass. The funnel then forms the external wall of the central cavity, its internal wall being formed by the central mass proper. The columnar cells of the tubule then continue with the epithelial ciliated cells of the funnel, gradually becoming transformed towards the border of the funnel. In a central cavity of this description the internal wall can be very irregular, with no special cells forming it. The cavity may be empty or it may contain more or less free cells, lymphocytes and phagocytes. In young specimens only one tubule (or two) opens into each cavity, but in older ones more than one or even many tubules may belong to one cavity, which may then be very large. Very rarely however, two neighbouring cavities join. These different conditions will be explained further on. Only in a few cases have I found the central cavities in open communication with the pronephric vein. Generally this condition is connected with a certain activity of the central mass. Such a condition occurs more or less regularly only in very old animals, in which the pronephros is degenerating. This is an important difference with regard to the conditions in *Bdellostoma*, where almost every compartment of the central duct is said to have an outlet through the central mass into the pronephric vein. When in *Myxine* a communication between the central cavity and the pronephric vein exists, this communication is not an immediate one, but has been effected by a central vacuity joining the central cavity and then

VOL. 31 195

Fig. 2. Pronephros of a 12 cm specimen (12.10). Fibrous type. Lettering as in fig. 1.

OL.
31
950

opening into the vein. As there is no endothelial layer covering the central mass, there is no endothelium lining the communication canal.

THE BLOOD SUPPLY OF THE PRONEPHROS.

For a long time past the fact has been well established that the pronephros becomes sunk into the pronephric vein, where its central mass comes immediately into contact with the venous blood stream. It is also well known that the tubules, at least basally, are surrounded by broad venous spaces or plexa communicating with the vein.

The supply of arterial blood of the pronephros of *Myxine* has been described in some detail especially by MAAS (1897). CONEL (1917), however, does not mention any arteries in the kidney of *Bdellostoma*.

According to MAAS, a number of segmentally arranged afferent arteries issue from the dorsal aorta entering the central mass of the pronephros, one for each compartment. In his diagram there are six such arteries present in the 8.5 cm stage. In the 9.8 cm stage their number has decreased to three. These blood-vessels are said to form a dense plexus in the central mass. In a reconstruction of the pronephros MAAS pictures three arterial vessels which *penetrate* the wall of the pronephric vein and *cross over its lumen* to reach the central mass ("strittige Gewebe" of MAAS). In this mass these vessels form "umspinnende Gefässe". MAAS seems to have overlooked the extremely improbable behaviour of these arteries, crossing the lumen of a vein.

In the hundreds of head kidneys I have studied, I have never found an artery entering in the manner described by MAAS, and I am bound to confess that I have not found a single case in which an arterial vessel could be traced into the

central mass. I am therefore obliged to deny its existence. Nevertheless I have traced two arteries to the head kidney of a 9.6 cm. *Myxine*, viz. a strong artery (branched) to the glomus, and a weak artery coming from a dorsal aortal branch and going to the posterior end of the pronephros. This latter artery did not enter the pronephric vein and could be followed into the connective tissue at the base of a tubule. It is very probable that this artery connects up with the venous plexus surrounding the basal parts of the tubules.

Some peculiarities concerning the blood supply will be dealt with further on.

THE GENERAL STRUCTURE OF THE CENTRAL MASS.

The descriptions of the central mass given by the different authors are very inadequate. Most of them, however, indicate that its structure is very variable, and it is impossible to gain any clear understanding of the real structure of the mass. The most exhausting description is that of CONEL, but even this description gives only a poor idea of the central mass, its structure and transformations. The authors seem to agree as to the formation of the central mass through the transformation of cells belonging to the external wall of the central duct, but how this transformation was effected is not clear from their descriptions. Fibrous cells, rounded cells of different sizes, small nuclei and large nuclei, connective tissue are all described, and so forth.

After studying hundreds of sectional series of animals of different sizes I have come to the conclusion that *the only tissue common to all central masses of animals larger than 9.6 cm is the fibrous tissue.* This tissue is generally disposed as nets with small or large meshes, or as fibrous strings. (Fig. 3.) The meshes of the net are either empty or contain cells of different characters, small cells, cells of medium and large size and giant cells. Many of the cells are lymphocytes and phagocytes. Moreover, red blood corpuscles may be present in the network. The meshes of the net are of very different width. In old animals they may be very wide, many times filled with degenerating tissue elements. In the following pages I shall try to give a description of all these elements and also attempt to place them in relation to one another.

Degeneration and regeneration are regular occurrences in the central mass and as will be shown later on these occurrences are regularly repeated throughout life in a certain rhythm, indicating a functional periodicity of the head kidney.

The central mass of *Bdellostoma* is said by CONEL to be enveloped by the same endothelial tissue as forms the endothelium of the pronephric vein. This endothelium is also said to clothe the canals joining the vein lumen with the central duct. In *Myxine*, MAAS illustrates the vein endothelium as a layer covering also the central mass. Such a condition would unquestionably be very natural. When the pronephros sank into the wall of the vein it must have

VOL. 31 1950

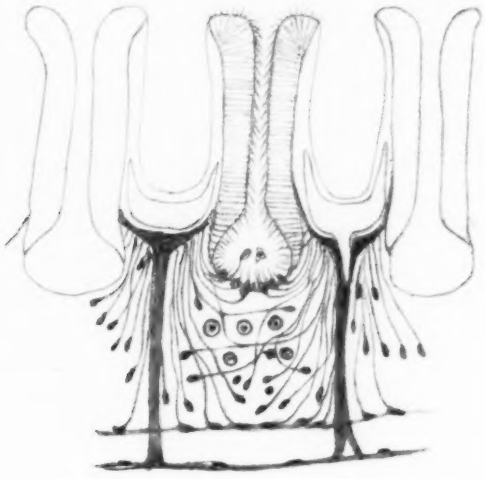


Fig. 3. Diagrammatical representation of the fibrous tissue of the central mass. In the meshes of the fibrous tissue are blood elements enclosed. The central mass is delimited by fibre cells forming a superficial layer. Two vertical trabecles.

pushed the wall of the vein before it, so that it became covered with the vein wall. But in *Myxine* there is no such endothelial layer on the surface of the central mass. Probably there may have once been such a layer, but already in my 9.6 cm stage there is none, and there is none in any of my hundreds of kidney specimens. From this statement the conclusion must be drawn that either there never was such a layer or that it became reduced very early. Thus the tissue of the central mass lies uncovered in the blood stream of the pronephric vein. In many cases the fibrous tissue forms a superficial layer, causing the central mass to have a good delimitation towards the vein lumen, but in many cases there is no limitation at all, cells contained in the external meshes of the fibrous tissue becoming free in the blood stream. These conditions will be treated later on in this paper.

Especially KIRKALDY (1894) and CONEL (1917) have pointed out that there is a considerable amount of connective tissue in the pronephros of *Myxine* (less in *Bdellostoma*). This connective tissue partly surrounds the tubuli, partly forms trabecles (Fig. 3) issuing from the angels between the tubuli and traversing the central mass and the pronephric vein, finally being inserted in the vein wall. As is seen from a picture in KIRKALDY's paper these trabecles may be followed by a strand of the central mass tissue down to the vein wall. I can confirm these results, but shall later on go into some questions related to these connective tissue trabecles. I shall here only state that they are built up of collagenous fibrous tissue.

THE PRONEPHRIC TUBULES.

The general arrangement of the tubuli is well known through the works of MAAS and CONEL. The description of their histology by the different workers does not differ very much. The tubuli are composed of a columnar epithelium in which the ovoid nuclei are generally said to lie at the same level in the cells. The cells are said to be sometimes ciliated, sometimes not. The difference in the descriptions is no doubt due to the state of the material used. However, it may be said to be an established fact that the cells carry ciliae. According to CONEL, each cell (in *Bdellostoma*) has a single strong flabella-like cilium. KIRKALDY illustrated a section of a tubuli with modified tubular epithelium, where there are two big rounded cells among the columnar cells. The openings of the tubuli into the pericardial cavity are of two different kinds: the epithelium of the tubuli ceases immediately at the pericardial endothelium or it bends outward, forming an epithelial funnel or rim around the opening before joining the pericardial cells. The inner part of the tubule opens into a central cavity, which has a very variable appearance, of which more will be said later on.

VOL. 31 1950

My researches have given the following results regarding the histology of the tubuli. The epithelium consists generally of columnar ciliated cells. In young animals their basal surfaces rest upon a very thin collagenous membrane ("basal membrane"). In older specimens this membrane may be rather thick. The thickening of this membrane is largely due to the fact that there is added to it apically a collagen component derived from the pericardium, and basally a similar component derived from the collagen tissue of the venous spaces between the tubuli. The nuclei of the columnar cells are generally arranged in a single layer, basally in the cells, but as they are rather long ovoid bodies their external ends reach about to the middle of the cell body. There are, however, differences in the height of the cells and also in the length of the nuclei. The form of the cells varies somewhat. They are, however, mostly broad basally and on a level with their nuclei, and narrow apically to the nucleus, widening somewhat at their apical surface, which forms a little knoll entering the lumen of the tubule. In preparations stained according to the Azan-Mallory method, this knoll stains somewhat darker than the rest of the cytoplasm of the cell. A close investigation of this knoll in material preserved according to Champy and stained with the iron-haematoxyline of Heidenhain (and 1350×magnification) reveals that it contains a superficial layer of small darkly stained corpuscles—basal corpuscles of the cilia. There may be 10—12 or even more such basal corpuscles on each knoll. The presence of these corpuscles makes it clear that there is a fairly large number of cilia on each cell and not, as has been assumed earlier, merely one. In most preparations, however, the cilia are joined together to form bundles suggesting flagellae. In especially well preserved pronephroi the very fine cilia are free. I am of opinion therefore,

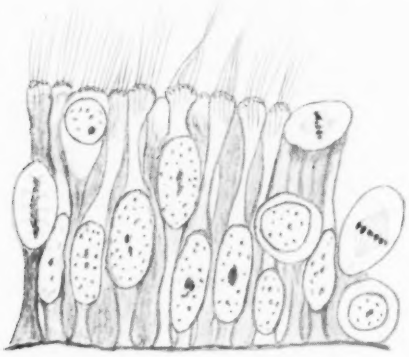


Fig. 4. Columnar epithelium. In the layer of columnar cells are rounded cells enclosed. Two of these are seen in mitosis. The rounded cells are transformed columnars.

OL.
31
950

that the normal state of things is that the ciliar apparatus is of a nature akin to free cilia, 10—12 or more on each cell. Sometimes I found a suggestion of a striation of the parietal cytoplasm issuing from the region of the basal corpuscles, directed towards the nucleus. The striation probably signifies the presence of ciliary roots. (Fig. 4.)

The columnar epithelial cells—in life—probably fit closely against each other, but in the transverse sections there seems to be a minute space between them. In the apical part of the cells this space is broader and fine lamellae are then seen connecting the cells with one another. These connections are probably artefacts or "Kittlinien." They are seen in longitudinal sections as a weakly stained space between the more darkly stained cytoplasma of the cells.

In the preceding lines the "normal" conditions of a tubule are described, but there are many other dispositions present in the tubules. Thus it is a very common feature that the cell nuclei do not lie at the same height in the cells. In such a case the cell body is differently shaped according to whether it belongs to basal or to apical nuclei. In the "basal cells" the base of the cells is broad, but the apical part very narrow, only widening at the tip, in the "apical" cells the base is very narrow, the apical part broader. Both classes of cells reach to the basal membrane as well as to the surface of the tubular canal. Such a disposition is common in tubules which are growing in length and is probably caused by the orientation of the mitoses, which, sometimes at least, are disposed obliquely to the longitudinal axis of the tubule. I do not doubt but that the uneven disposition of the nuclei indicates that growth of the tubuli is going on and that the cells will ultimately assume the "normal" position. Cells with a round nucleus may also be present. They may be disposed basally or apically (Fig. 4). These basal cells probably do not reach the lumen of the tubule, nor the apical cells not the base. The latter have cilia as normal cells.

I have seen cell divisions—in typical tubules—only in apical cells. The mitoses are there located parallel with the longitudinal axis of the tubule. When the mitosis is found in the most apical part of the tubule, this may possibly have something to do with the outbending of the tubule to form its rim. Other dispositions of the tubuli will be considered together with the transformations of the central duct and mass.

Degenerating cells are intermingled between the normal cells. In such cells most of the chromatin of the nucleus is collected along the central axis of the nucleus, there forming a dense string. This chromatin mass is connected with small chromatic corpuscles at the nuclear membrane by means of fine, somewhat chromatic, strings (Fig. 4), so that it seems as if the central part was surrounded by a layer of vacuoles. The cytoplasma of such cells by degrees becomes basophilous. In some cases I have also found an apical degeneration of tubules. In such cases it seems to be the old and long tubules that are subjected to degeneration. Mostly such tubules open direct into the pericardial cavity without a rim or with a poorly developed rim. In the degenerating part the columnar cells are translucent, blown up to form rounded bodies, in which the disfigured nucleus has lost most of its staining property. Or else the cells have preserved their shape, but become translucent. Such degenerating cells at the opening of the tubule seem to leave the epithelium and get free into the body cavity as small vesicles containing a fluid. Around the tubular opening the cells of the pericardial wall may also be transformed into similar vesicles.

But a degeneration may likewise occur in tubules with a mouthpiece. In that case the degeneration begins with the mouthpiece, where the cells lose connection with one another and come more or less freely into the pericardial cavity. The openings of such tubules look as if they were surrounded by a brush of threadlike, more or less discoloured, cells, in many of which the nucleus could no longer be recognized. The columnar cells of the tubules also sometimes show a distinct degeneration as they seem to have lost their connections with one another. This appearance is no artefact, as healthy tubules are present in the same sections lying immediately by the side of the unhealthy ones. This form of degeneration seems to be located especially at the posterior end of the pronephros and to break in upon an entire system of tubules.

Another sort of degeneration is sometimes met with in comparatively young as well as in old pronephroi. This mode of degeneration begins apparently basally in the infundibulum and seems to spread to the base of all the tubuli which open in this infundibulum. Central cavities belonging to such infundibula are, partly at least, cut off from the rest of the pronephros by means of a thick collagenous wall or a system of collagenous trabecles, which seem to prevent the degenerating cells from leaving the compartment in the direction of the central mass, where degeneration also takes place. They lose their connections with one another, coming out in the lumen of the tubuli. They are now strongly

VOL. 31 1950

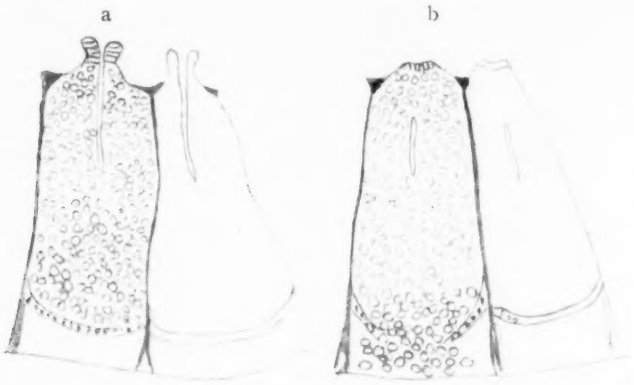


Fig. 5. Reduction of tubuli through excessive development of haemocytoblasts. Diagram: a) stage with tubules still present. b) with tubules reduced to a mouthpiece. Central mass bursting. The original central mass reduced to a thin layer, delimiting the mass from the pronephric vein.

basophilous. They are apparently caught by the stream produced by the ciliae of the still undegenerated more apical cells of the tubule. This stream, which is generally directed basally, apparently is directed apically in the degenerating tubule, so that the degenerating cells are ultimately passed on to the pericardial cavity. Similar degeneration occurs sometimes where the degeneration begins at the end of the tubuli, but I could not follow the stages of this process, which, however, seems to proceed in a basal direction.

Another form of elimination of tubuli seems to be quite normal. It occurs when practically all the columnar cells suddenly begin to divide mitotically, producing such a mass of rounded elements that the canal of the tubule becomes obliterated (Fig. 5). This big cellmass is then added to the central mass, the tubule disappearing completely, leaving only its mouthpiece intact. From the mouthpiece a new tubule is regenerated. As this kind of elimination was found in head kidneys kidneys belonging to animals of different sizes, it seems to be a normal occurrence.

THE FORMATION OF NEW TUBULES.

PRICE (1910) says that in *Bdellostoma* new tubules "branch out from the old tubules just back of the nephrostome, and while still very short acquire an opening into the pericardial cavity." "It seems certain that new tubules never grow out from the central duct." CONEL also seems to be of the opinion that new tubules arise as branches of older ones. Another mode of development is mentioned in the literature (MAAS, 1897), viz. where the tubule takes its origin in the pericardial epithelium.

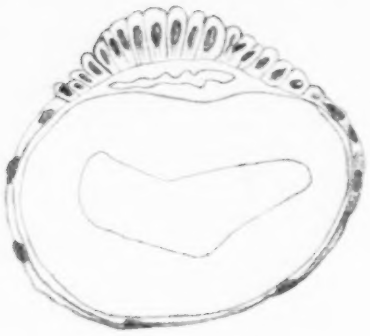


Fig. 6. Initial stage of the development of a new tubule from the pericardial epithelium.

Fig. 7. Further development of a tubule in the pericardial wall. Later on the rudiment will be invaginated in the centre to form a new tubule.

VOL. 31 1950

I have had ample opportunity of studying these two modes of origin and, in addition, a third and a fourth, which will be described below.

As already stated by PRICE, the new tubules mostly develop near the tip of old tubes. There arises a hollow outgrowth of the columnar cells of a pre-existing tube. This outgrowth is surrounded by the thin collagenous basal membrane of the tubus. It very soon reaches the basal membrane of the pericardium. At this place the pericardial epithelium is somewhat thickened with more densely arranged nuclei. The outgrowth here breaks through to the pericardial cavity and opens into it, piercing the modified pericardial epithelium. The opening of tubules formed in this way is generally narrow. It is surrounded by somewhat thickened pericardial cells, but without a special mouthpiece.

The initial stage of a tubule forming out from the pericardium is a thickening of the pericardial epithelium. These thickenings are mostly situated near the tip of a pre-existing old tubulus. The cells of this thickening are cylindrical. The middle portion of such a thickening is then cut off from the rest, its cells becoming higher (Fig. 6). As the cells increase in height the rudiment rises up above the surface of the pericardium, forming a round disc, (Fig. 7) consisting of more or less columnar cells. A vivid cell proliferation causes the disc to grow out to form a substantial cushion, consisting of threadlike cells with the oblong nucleus lying at different heights on the threads. The cells widen apically to a small superficial knoll, which seems to carry cilia. The basal end of the thread joins the underlying collagenous connective tissue. Sometimes expansions of blood-vessels were found below the middle of the cushion. A step further in development reveals the cushion with a central depression, a depression which later develops into a tubular invagination, which grows

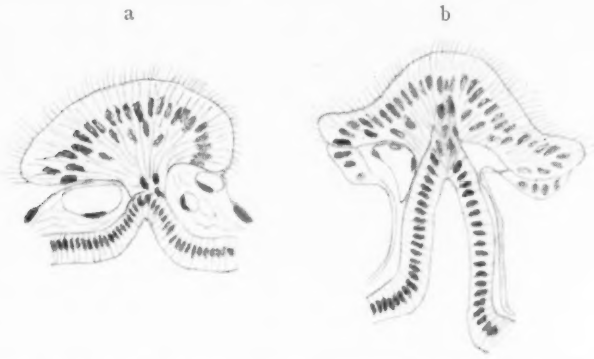


Fig. 8. Development of a new tubule from the pericardial wall and a preexisting tubule. a) early stage. b) the tubule breaks through the pericardial rudiment.

inwards into the pronephros. It is in this way that most tubules with a mouthpiece arise.

The third way for a new tubule to develop is a combination of the two already described. A tubular outgrowth from a pre-existing tubule reaches the middle of a rudiment of a pericardial tubule (nephrostome) (Fig. 8a). Where the tubule reaches the nephrostomal rudiment, cells at its tip enter the middle of the rudiment, forming a conical plug, which breaks through the rudiment (Fig. 8b). This plug becomes hollowed out, so that the tubule gains an opening in the middle of the nephrostomal rudiment. Tubes with mouthpiece may thus have a double origin.

In half-grown and older animals tubules may arise also from the central mass. In such specimens epithelial alveoles are formed through cell bodies belonging to fibre cells arranging themselves around a small space, generally containing a degenerating cell. Such alveoles sometimes grow larger, become tubular and grow out to the pericardium, forming a new tubule. In other cases bodies of fibre cells form an epithelial disc, usually in connection with a trabecle. This disc becomes hollowed out on its deeper side, where a central cavity arises; the hollow deepens to a tube, which ultimately connects with the pericardium. But most of these bigger alveoles join neighbouring infundibula, making them wider.

Sometimes rudiments of nephrostomes fall into decay before they have joined on to the tubule. In such cases there arise very peculiar pictures. From a centre, consisting of a collagenous mass, issue radially loosely connected clublike cells, containing a degenerating nucleus at their distal swollen end. In rudiments where the decay has not yet advanced very far healthy cells are contained in the interior of the cushion, whereas the external portions are degenerating. The end of the process is reached when all cells are so strongly

transformed that no definite structure can be recognized in them. They are then reduced to vesicles, which are delivered to the body cavity. This sort of degeneration was found in portions of the pronephros where degeneration of the tubules begins with the mouthpiece as described in preceding pages.

THE FIBROUS TISSUE OF THE CENTRAL MASS.

Most investigators of the head kidney in Myxinoids have spoken of the fibrillar appearance of the central mass, but its real nature has not as yet been fully recognized. KIRKALDY, however, says that some of the columnar cells of the tubules become transformed into fibre cells.

In preceding pages it was stated that the columnar cells of the tubules rest upon a basement membrane. This membrane is composed of collagenous matter. In the youngest specimens it is very thin, but in older ones becomes considerably thicker, probably owing in part to the activity of the columnar cells. Basally this membrane passes from one tubule to another. It covers that widened funnellike part of the tubule which forms the external wall of the central cavity (duct), but where this wall bends inwards the basement membrane becomes detached from it and passes over to the next tubule. (The basement membrane thus does not cover the entire central duct. [CONEL, 1917].) At this point the basement membrane lies immediately upon the central mass, of which the superficial cells have in very young animals an indifferent appearance but in older ones assumes a special character, as will be described below. The columnar cells of the basal part of the tubule have generally an attenuated basal portion. Towards the border of the basal funnel this condition is more accentuated, the basal attenuated portion being strongly prolonged. Some of these transformed columnar cells bend towards the central cavity, and that part of their cell body which contains the nucleus and carries the ciliar apparatus forms the epithelium of the internal wall of the cavity. This internal wall—while still epithelial—also consists of similar cells which have apparently lost their basal prolongation, and thus their connection with the basement membrane. Other cells at the basis of the tubule (at the angle between two tubules) extend into the central mass without any connection with the central cavity (Fig. 9). They have a variably prolonged attenuated basal part, somewhat conically widened where it carries the nucleus and a small amount of cytoplasma surrounding it. The length of the attenuated part is very variable. There are cells with rather short and others with very long basal part, probably owing to the degree of development of the cells. Such attenuated portions of the transformed epithelial cells form the fibrous tissue of all head kidneys in *Myxine*. The fibres of such cells are so disposed that they together form an alveolar structure with alveoles of different width, which may contain blood

VOL. 31 1950

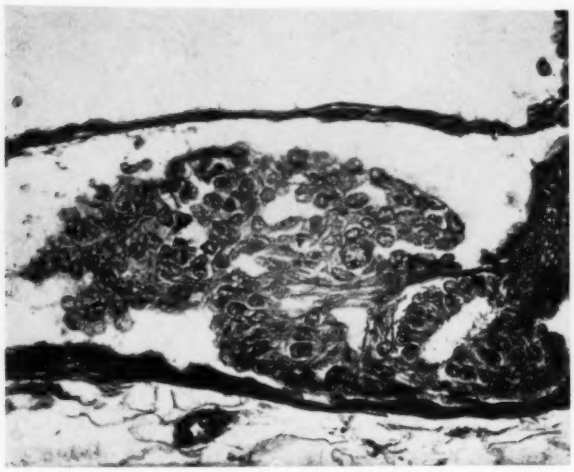


Fig. 9. Pronephros 12 cm specimen (12.9). Fibre cells, developing at the base of a tubule.

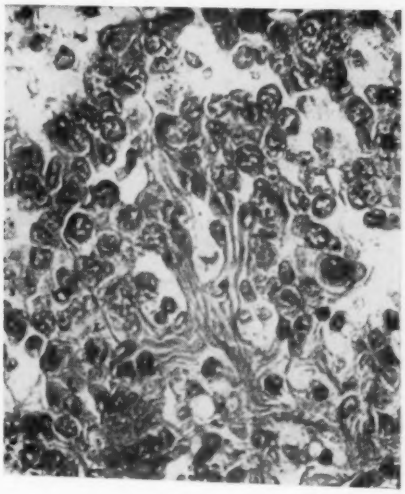


Fig. 10. Development of the fibre capsules in the central mass in a 19 cm specimen (19.1). In one of the alveoles two lymphocytes and a phagocyte are seen.

cells of different kinds (Fig. 10). These alveoles are not closed all round, but may communicate through openings of very variable dimensions.

This disposition of the thread cells to form incomplete capsules around blood elements in the central mass is not always possible to demonstrate. Frequently it seems that real capsule cells may possibly be present. When blood elements are produced in great numbers in the basal parts of the tubules, adnexed columnar cells lose their character of such cells and are transformed into cells surrounding blood elements more or less perfectly (Fig. 11). It is not possible, however, to tell for certain that such cells really form capsules even if they appear to do so. In other instances blood elements may be enclosed in capsules the nature of which, owing to transformation of fibre cells, cannot be stated.

The nucleus of the capsule cells is, if viewed from the surface oval. From the side view they are more or less triangular with a flat surface following the inner outline of the capsule and a convexity on the opposite side. Usually these cells stain darkly, like the nuclei of the fibre cells. The size of these cells is the same as that of the cell-bodies of the fibre cells. They may therefore be transformed fibre cells.

The fibre cells are liable to undergo many transformations according to the functional state of the kidney. These transformations will be treated further on. To what has already been said about the general appearance of this cell type, we need only add here that the fibrous part of the cell is cylindrical, with a

Fig. 11. Columnar cells transforming to capsules around haemocytoblasts in tubules of a 10 cm specimen (10.5). The capsule-forming columnar cells are seen especially in the central and the left tubules.

VOL. 31 1950

glistening and very smooth surface. "Normally" such threads, lying close together, do not adhere to one another but retain their independence. The nucleus is "normally" somewhat longer than broad, containing a network with many dark-staining chromatic corpuscles. The threads of the cells in sections stained with iron-hematoxyline are unstained. By contra-staining with Hansen's picro-fuchsin the threads assume a very pale pink colour. The basal end of the thread seems always to be related to a collagenous basal membrane or a collagenous layer or trabecle. Where the thread is joined on to such collagenous structures it is clearly seen that it continues into the collagen mass. At least the main part of the collagen of the kidney consists of the most basal parts of threads. This part is transformed into collagen. Therefore I believe that the rest of the thread contains some precollagenous matter.

It has already been stated that in very young animals there are cells of indifferent appearance present in the space between two neighbouring tubules. In older specimens such spaces frequently become wider. There the indifferent cells are transformed into an epithelial-like cell layer, consisting of cells being transformed into short-thread cells, which later grow into long thread-cells, and cells developing into blood elements (haemocytoblasts).

THE COLLAGENOUS STRUCTURES OF THE HEAD KIDNEY.

It has been stated by the different authors that there is a considerable amount of connective tissue present in the head kidney of Myxinoids, *Myxine* having more of it than *Bdellostoma* (CONEL, 1917). It seems uncertain, however, whether this connective tissue does not comprise also the fibre cells and not only the collagenous structures. It is, however, well known that the tubules are surrounded by connective tissue and that the central mass is penetrated by connective tissue bands or trabecles joining the wall of the pronephric vein.

The general arrangement of the collagenous structures, as they appear especially in young animals, is the following.

Below the pericardial epithelium a collagenous layer of variable thickness is developed. In young animals it is rather thin, in older ones it becomes stronger. The pronephric vein is provided with a strong collagenous layer outside its endothelium. Towards the border of the pronephros this collagenous layer connects with that of the pericardium so intimately that the two layers cannot be separated from one another. This joined layer, covered on the outside by the pericardial epithelium and on the inside by the endothelium of the pronephric vein, connects with the pronephric tubules. There the collagenous layer connects with the basement membrane of the columnar cells and with a thin collagene membrane outside it. Inside this follows the endothelium, which may be followed along the outside of the tubule to its base. In the triangular space (in young specimens) between two tubules is enclosed a venous space covered by the endothelium, and outside this by a thin collagene membrane, the basement membrane and the collagene of the pericardium of the tubule. Around the bases of the tubule the collagene membranes are thickened so that a ring of collagene is formed surrounding the bases (Figs. 1 and 3). In older animals these latter membranes are thicker and the rings are strengthened considerably through bundles or balks of collagene substance. Generally the pericardial membrane is folded in between the tubules, so that the venous spaces are pressed together between this membrane and the membranes surrounding the tubules. In older animals connections are formed between these different membranes, so that the venous space is even more reduced. Especially between the bases of the tubules the collagenous matter has increased considerably and connections have been established between the basal rings and the pericardial collagene, the venous spaces becoming reduced to narrow tubes—i.e. to a blood plexus. Through this process there thus arises a secondary system of venous vessels surrounding in particular the bases of the tubules but also reaching up towards their tips (Fig. 3). There is great variability in the development of the collagene structures. In old animals they are generally very strong, but old animals are also found in which they are not considerably more developed than in young ones. Old animals with

small pronephroi generally also have poorly developed collagene. This seems to be due to the occasional destruction of the collagene.

A very characteristical feature of the pronephroi is the presence of the collagene trabecles (Figs. 1, 3), which traverse the central mass and the pronephric vein, being inserted on the inside of the latter. The occurrence of these trabecles is, however, variable. In small animals they may sometimes be absent, but when present their number is very variable. The same is also the case in bigger specimens. These penetrating trabecles are disposed in a variable manner. Some of them have a horizontal course connecting the collagene rings around the tubules with the venous wall, others are more perpendicular. The trabecles may frequently, especially in old animals, be branched in different ways. They may be connected with one another by means of strong horizontal bridges, and so forth.

In young animals the trabecles consist of one or a few collagene rods issuing from the angle between two tubules. These rods are surrounded by some mostly flattened cells, scattered in a thin layer of connective tissue. In some cases, however, I have observed the proximal end of such trabecles surrounded by fibre cells, of which the basal part is connected with the connective tissue layer of the trabecle, whereas the opposite part reaches a central cavity.

To understand this structure it seems advisable to investigate what structures are present in the angle between two tubules (Fig. 3). Beginning from the pericardial side, we first meet the endothelium of the venous space between the tubules; then follows the collagene mantle of this space with its scattered connective tissue cells and, finally, the basement membrane of the transformed columnar cells between the two tubules. These cells are either low epithelial-like cells or already young fibre cells. When a trabecle forms at the angle, the only collagene structures which can take part in the formation of the trabecle are the collagene mantle of the venous space and the basement membrane of the fibre cells. From the collagene layer a rod now grows out into the central mass, carrying with it the scattered connective tissue cells, which thus form a layer outside the compact collagene rod. This outgrowth now meets the basement membrane of the fibre cells and carries it into the central mass. In this way a trabecle is formed, consisting of a central rod, surrounded by a thin layer of connective tissue and by fibre cells with their very thin basement membrane. As the rod thickens, the connective tissue cells become embedded in the rod, and the latter comes into contact with the fibre cell layer.

I believe therefore, that the matrix cells of the trabecle are derived from columnar cells at the bases of the tubules. An additional argument for this conception is the statement that these trabecles augment their calibre through the activity of typical fibre cells, which may be seen dipping the ends of their fibres into the surface portion of the trabecle, in transverse sections appearing

VOL. 31 1950

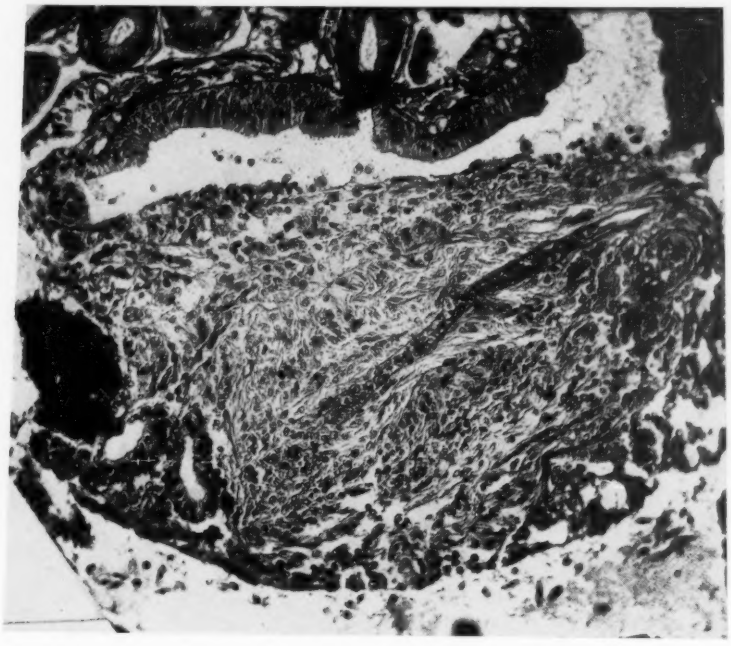


Fig. 12. Trabecular blood-vessel in compact central mass of a 33 cm specimen (33.9). This long vessel is filled with lymphocytes of an unhealthy appearance.

as if they were radiating from it. The basal portion of the fibres in that case is collagenous.

Trabecles of this description are also common in older animals. But, besides these, another type is frequently met with. In that case the trabecle consists of a number of collagene rods between which are clear (venous) spaces. The entire collagene matter may also be surrounded by a clear space. These spaces are covered by very thin membranes consisting of endothelial cells. It has been stated that in many cases these spaces are continuous with the venous spaces contained in the basal collagene rings of the tubules.

The development of this type of trabecle is easily understood, issuing from the type already described. From the venous space between the tubules the endothelium sends forth a venous pocket into the young trabecle. This pocket penetrates into the trabecle between it and the basement membrane of the fibre cells. As the collagene rod simultaneously thickens, the pocket may become surrounded by collagene and surround the collagene rod. Simultaneously the bases of the fibre cells are transformed into collagene, and so a trabecle of the type just described has arisen.

Lymphocytes and even red blood corpuscles have been found in the trabecular

Fig. 13. Young blood sac in a 27 cm specimen. It is seen in the centre of the central mass. It contains a few blood elements and transsected trabecles.

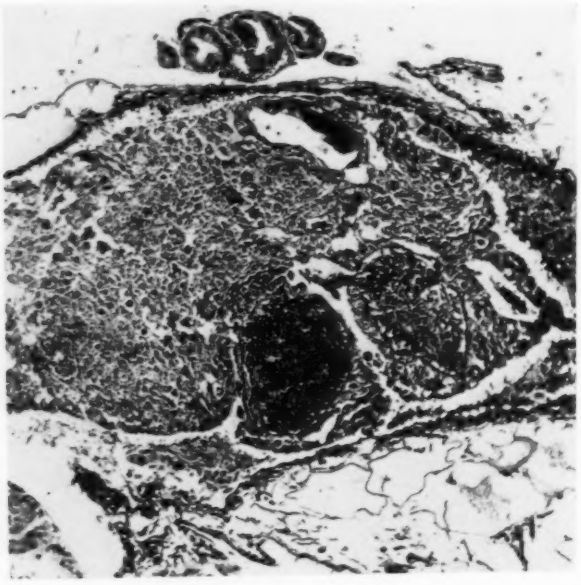


Fig. 14. Blood sacs in a 33 cm specimen (33.6). See text.

VOL. 31 1950

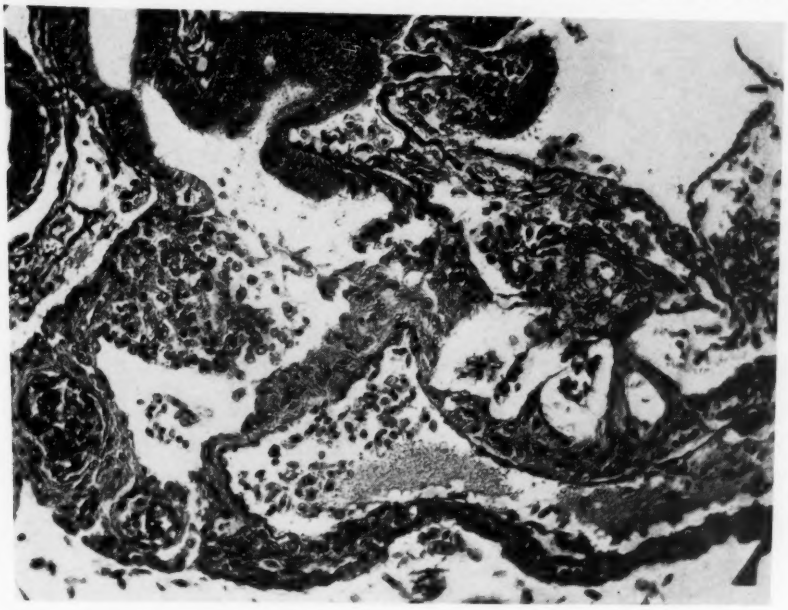


Fig. 15. Very large blood sac in a 33 cm specimen (33.4). Essentially fibrous type. The blood sac lies basally in the central mass near its border. Dorsal to it lies a big trabecle with fibre cells radiating from it. The sac contains blood elements and coagulated matter.

spaces, which may thus be considered to act as a sort of blood-vessels. In older animals (for instance of 33 cm body length) such trabecular spaces can emit real vessels (Fig. 12) entering and branching into the central mass. As far as that can be stated with any certainty, such blood-vessels end in the alveolar structures of the mass. These vessels may be empty or contain lymphocytes or red blood corpuscles or both. These vessels are provided with a thin endothelium, and outside this there is a thin collagene layer which may contain thickened collagene bands. In their interior also collagene threads or rods are sometimes seen, which suggest that the vessel has arisen in connection with a trabecle.

Sometimes these spaces are widened so as to form sacs of different dimensions (Fig. 13). Such sacs are then delimited by an endothelial layer, and outside this a collagene membrane, to which fibre cells are fastened with their fibre ends. These cells apparently produce this membrane. The fibres of these cells (when the sac is much expanded) are pressed against the thin collagene layer of the sac and form an external covering of the sac. In sections tangential to the sac it is clearly seen that the fibres are parallel with each other and directed mainly towards the trabecle to which the sac belongs. The cell bodies belonging to the fibres are found around the sac. Sometimes blood elements

Fig. 16. Pronephros of a 19 cm specimen (19.1). Collagen thread or rod in a central cavity. Detritus masses (decaying lymphocytes) in the pronephric vein (to the left).

are formed between the fibre cells. The trabecular rod is generally present in the interior of the sacs. The smaller sacs are usually empty, but the larger ones contain blood cells of different appearance, as will be described later on. These sacs I shall call "blood-sacs" (Figs. 13, 14, 15).

In addition to the two types of trabeculae already described there is a third. In this case the trabecle arises without any connection with the venous spaces somewhere in the midst of the central mass or in a central cavity (Fig. 16). These trabecles are simple collagenous rods surrounded by fibre cells, of which the fibres dip into the collagene of the rod, augmenting its volume.

In some cases the trabecles seem to expand their collagene structures into the central mass, forming a network of fibres or lamellae (a sort of reticular connective tissue). This case is especially met with in kidneys in which the production of blood elements is very vivid (Fig. 70).

That part of the trabecle which connects with the wall of the pronephric vein is clad with endothelium from this vein. The venous vessel of the trabecle never joins any blood-vessel outside the pronephric vein.

THE CONTENTS OF THE BLOOD SACS.

The blood sacs first appear when the specimen has attained a length of 27 cm. In specimens of that size the contents of the blood sacs are somewhat different.

VOL. 31 1950

For instance: in the head-kidney illustrated (Fig. 13) the blood sac is very nearly empty. In this picture transections of the trabecular rod are seen in the bottom of the sac and in its nec portion. A few red blood corpuscles and lymphocytes form the only contents of the sac. In most cases, however, the blood sacs are filled up with lymphocytes, in some with red blood corpuscles. Sometimes both red and white corpuscles are present.

In another kidney from a 27 cm specimen a spherical blood sac is present. It is filled with densely packed lymphocytes. In the centre of this mass of lymphocytes is a rather big clear space, containing large phagocytes and degenerating nuclei of lymphocytes, some of them enclosed in the cell body of phagocytes. The lymphocytes surrounding this clear space are more or less degenerating. Many of the nuclei are dividing amitotically, and cells with two nuclei pressed against each other are common. The phagocytes have a big, pale nucleus. Their cytoplasma is finely granulated, containing big vacuoles in which degenerating nuclei of lymphocytes are enclosed.

In a specimen of 33 cm body length the head kidney is provided with a number of blood sacs of the type just described, but besides these there is a very big blood sac (Fig. 15). This sac contains a number of red blood corpuscles and some lymphocytes and spindle cells. It has a wide opening into the pronephric vein, through which it may have obtained its contents. At one time the sac was probably filled with blood elements of some kind, which, however, have left it, and it is now filling again with blood.

In another specimen of 33 cm two big blood sacs lie close together (Fig. 14). The one of them is filled with densely packed red blood corpuscles, with rather dark granulated cytoplasma and unhealthy-looking nuclei. The latter are clearly degenerating. Some pale-coloured nuclei, larger than the nuclei of the blood corpuscles, probably belong to phagocytes dispersed among the blood corpuscles. In addition, small groups of degenerating lymphocytes are present. The other blood sac also contains red corpuscles, but these are not especially densely aggregated and do not seem as yet to be very degenerated. In this blood sac there is an upper partition, where the content is quite different, there having developed—in connection with the trabecle—a sort of collagenous reticular connective tissue, in the meshes of which blood elements—proerythrocytes and lymphocytes, are enclosed. This partition is situated inside the capsule of the other part of the blood sac. It may represent a kind of lymph nodule.

The fate of the blood sacs is obscure. Probably they must burst and deliver their contents into the pronephric vein. Evidence as support of this assumption is the masses of degenerating lymphocytes and erythrocytes which are sometimes found in the pronephric vein, and which can scarcely have any other origin than from blood sacs (Fig. 17). Similar masses are also found containing degenerated red blood corpuscles.

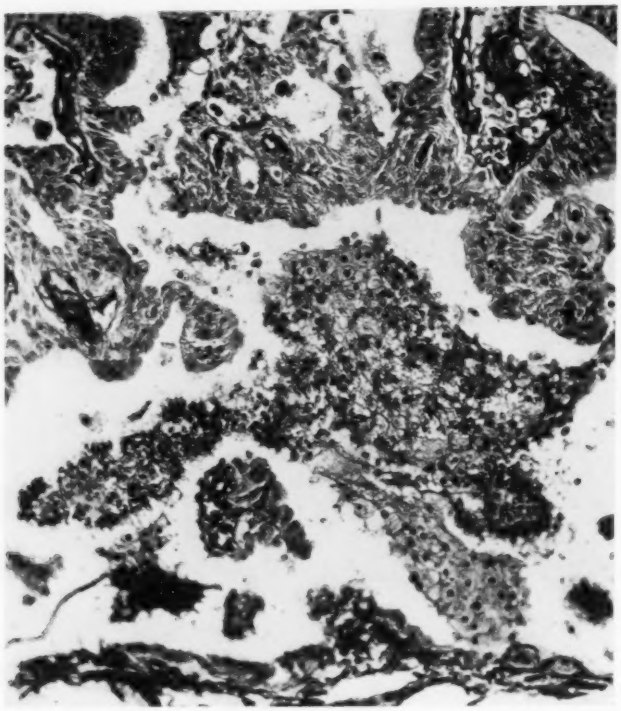


Fig. 17. Pronephros of a 33 cm specimen (33.5). Large masses of decaying lymphocytes and erythrocytes, probably from a burst blood sac, in the pronephric vein. Fibrous type.

VOL. 31 1950

The conclusion regarding the function of the blood sacs is that they are organs for collecting and destroying blood elements, especially lymphocytes and red blood corpuscles.

EPITHELOID ALVEOLES IN THE CENTRAL MASS.

Epithelial-like structures are often found (from the 25 cm stage and onwards) embedded in the central mass. In preceding pages it has been stated that epithelial plaques, formed by the cell bodies of fibre cells, may be initial stages leading to new tubules. Other such plaques are added to the rim of the infundibula, causing a widening of the central cavities. Many of the epithelial plaques form cuplike structures, in their hollows enclosing a few fibre cells belonging to the central mass. The epithelial-like cells of these cups either are connected with their fibres or have lost connection with them. The nucleus of such transformed fibre cells is situated at the convex side of the "epithelium". On the concave side these cells soon develop a ciliary apparatus. Such plaques

or cups play an important part in the widening of the central cavities. The more regular occurrence of these structures begins when the specimen has reached a body length of about 30 cm. They have only been found to occur when the central mass is strongly developed, rich in fibre cells.

Closed aveoles are found in the 32 cm stage and onwards together with the structures mentioned in the foregoing paragraph. These alveoles are mostly small, otherwise built as the plaques or cups. Frequently the alveoles enclose a degenerating cell of unknown origin, but probably a fibre cell. In other cases the alveoles are empty. Small alveoles are frequently met with usually developed in fields at the the rim of an infundibulum, and containing a body of colloidal appearance. This body assumes a brownish red colour, when stained with Hansen's picro-fuchsin, but the colour is not that of the collagene in the same section. A thin layer on the surface of the body is darker than the centre of it and seems to be somewhat granular. Sometimes simular bodies are found between the cells of the alveoles. The "colloidal" bodies are delivered to the pronephric vein, where in some cases they were found just outside an alveolar field. Whole alveoles with their contents were also found swimming freely in the vein. In that case the alveolar cells were degenerating. As such "colloidal" bodies never were found in blood-smears, it is possible that they dissolve in the blood. Are these "colloidal" bodies the product of an incretory activity of the head kidney? Or are they collagene bodies, resulting from the activity of transformed fibre cells (cells of the alveolar wall) and destined to disappear from the kidney and dissolve in the blood? In both cases they may be of hormonal importance.

THE DISTRIBUTION OF THE FAT IN THE PRONEPHROS.

It has already been mentioned that the megacaryocytes of the head kidney contain fat drops along their margin. But this is not the only case where fat is present in the kidney. In the following lines some cases are mentioned in which fat has been found in the head kidney.

1. The normal columnar cells never contain fat. When, however, columnar cells are transformed into big round cells, haemocytoblasts, fat drops appear around their nucleus.

2. Haemocytoblasts and megacaryocytes always seem to contain a mantle of fat drops (Fig. 18).

3. Proerythroblasts have also been found to contain fat drops, and likewise in polychromatic erythroblasts a small amount of fat drops may occur.

4. In the cytoplasma of fibre cells small fat drops were seen, and also between the fibres of the "fibrous tissue".

5. Phagocytes in the central cavity and also in the central vacuities may contain fat, as was stated in a few cases.

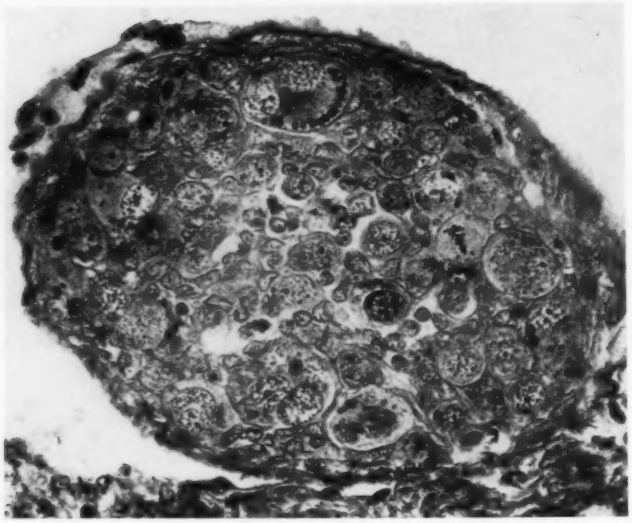


Fig. 18. Megacaryocytes, haemocytoblasts and their descendants in the central mass of the pronephros of a 12 cm specimen (12.8). Fat in the cells especially seen in the large megacaryocyte at the upper border of the central mass.

VOL 31 195

6. Once it has been stated that cells (proerythroblasts and polychromatic erythroblasts) containing fat are degenerating at the surface of the central mass, their fat contents being dispersed into the blood of the pronephric vein.

As the flowing blood of *Myxine* never contains cells with fat drops included it is obvious that the fat is either used up during the development of the blood cells in the pronephros or set free through the destruction of their cells. In the latter case free fat drops are met with, especially in the pronephric veins.

In one case I have found fat in the phagocytes of the glomus.

The distribution of the fat in the pronephros could not be studied in detail owing to a lack of properly fixed material. As I had only a comparatively small number of kidneys fixed with osmic acid containing fluids at my disposal, there are many gaps to be filled in by closer examination. Nor could it be determined what kind of fat is contained in the cells.

THE GLOMUS.

As already mentioned, the so-called Malpighian body of the head kidney develops into three pockets evaginated from the pericardial endothelium just behind the rudiment of the pronephros. In the bottom of each of these pockets a glomerulus arises. Then this complex of glomeruli sinks into a common pocket of the pericardial wall. This pocket never closes. Thus the glomeruli always

have free communication with the pericardial cavity. When this pocket forms, the mouths of one or two of the tubules lying next to the glomeruli are drawn into the pocket, so that the head kidney proper obtains direct communication with the glomerular pocket.

The Malpighian body is thus composed of three originally separate glomi and is a composite glomus lying in a part of the pericardial cavity. Its original position behind the pronephros soon alters to a position below the posterior part of it.

The glomus is a grapelike body, composed of rounded lobes, covered with the excretory epithelium derived from the endothelium of the pericardium. As I have shown elsewhere (HOLMGREN 1946), this endothelium is in some parts transformed into clublike excretory cells. Among the excretory cells of the glomus such clublike cells are very common, but generally the cells are nearly hemi-spherical. The excretory cells rest upon a collagenous layer, inside which the endothelium of the pronephric arterial convolute immediately meets it. The collagenous layer consists of threads forming a reticulum between the excretory cells and the endothelium of the artery. Between the meshes of this reticulum the excretory cells and the endothelium meet, a condition that facilitates the passage of excretory matter from the arterial convolute to the excretory cells.

OL.
31
950

THE EXCRETORY CELLS.

These cells are generally more or less half-spherical in transversal section with a flat base resting upon the collagene layer separating them from the endothelium of the convolute. The external outline is rounded because of the comparatively large nucleus protruding into the pericardial pocket. The cells form an epithelium. When the capillary inside the excretory layer widens, the cells become more flattened and separated from each other, their basal parts being drawn out into a thin flat foot-piece. Very frequently the cells are drawn out into perpendicular direction, so that they become pear-shaped and often even pedunculated. The stalk of the cell can be developed into a rather long thread (Fig. 19). In the excretory cells vacuoles are frequently discernible in the basal part of the cell inside the nucleus, and it seems possible that these vacuoles are due to some fluid passing from the capillary inside the cell and collecting in it.

In some cases some excretory cells are enormously developed. In fig. 20 such a cell is illustrated from a 35 cm specimen. It is a pedunculated cell, 70 μ long. This cell contains many darkly stained (iron-hematoxylin) somewhat oblong bodies filling up the cell, leaving a clear space near its outer surface. These bodies are, as far as could be observed, identical with the "yellow bodies" which are found in the epithelial cells of the mesonephric duct and must be excretory products. They are delivered to the pericardial pocket, where they

were found in another 35 cm specimen. In that specimen such bodies are found not only in the glomus but also in tubules and in phagocytes collected in the central cavity. It seems possible, therefore, to conclude that the bodies from the glomus enter the main pericardial cavity and thence through the tubules into the central cavity, where they are phagocyted. Some of the bigger bodies, however, only pass through the central body and come into the pronephric vein, where they are probably dissolved, as such bodies were never found in film preparations of the blood.

In the pericardial pocket in which the glomerulus is developed free cells are nearly always found. These are excretory cells which have been freed from the excretory epithelium. Many of them have already developed into phagocytes, others are like lymphocytes: They are either destroyed in the pocket or discharged into the pericardial cavity, whence they may enter the tubules and reach the head kidney.

VOL. 31 195

THE COLLAGENOUS STRUCTURES OF THE GLOMUS.

As already stated, this layer is not a continuous sheet like a basal membrane, but is composed of collegenous threads forming a fenestrated membrane between the excretory cells and the convolute. This collagene layer is the direct continuation of the collagene connective tissue of the pericardial wall, and the afferent artery of the glomus takes apparently no part in the formation of it, as this artery when entering the glomus is a simple endothelial tube.

THE CAPILLARY CONVOLUTIONS OF THE GLOMUS AND THEIR CONTENTS.

The endothelium of the capillary convolutions is generally very thin, with flat cell nuclei. In young specimens these cells are generally thicker with the nuclei bulging slightly into the capillary.

In many convolutions, especially in large specimens, besides the endothelial cells a layer of large, almost cubical cells is present. These cells almost suggest an epithelium inside the endothelium (Fig. 21). The comparatively small nuclei of these cells are irregularly shaped. The cytoplasma contains a great number of coarse dark-stained granules. In one case fat drops were also found in these cells. Some of them were basally constricted and others had lost their connection with the endothelium, lying free in the capillary. Such free cells were found in the efferent artery and are consequently poured out into the blood. The big cells are a kind of phagocytes, which have absorbed and stored up matter perhaps being conveyed with the blood to the glomus. When the supply of such phagocytes is large, the capillary convolution is strongly inflated. The

Fig. 19. Club- and fibre-like excretory cells dorsally in the glomus of a 19 cm specimen. The large vesicles are strongly dilated capillaries of the glomus.

OL.
31
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Fig. 20. Excretory cell with enclosed "yellow" bodies from the glomus of a 35 cm specimen.

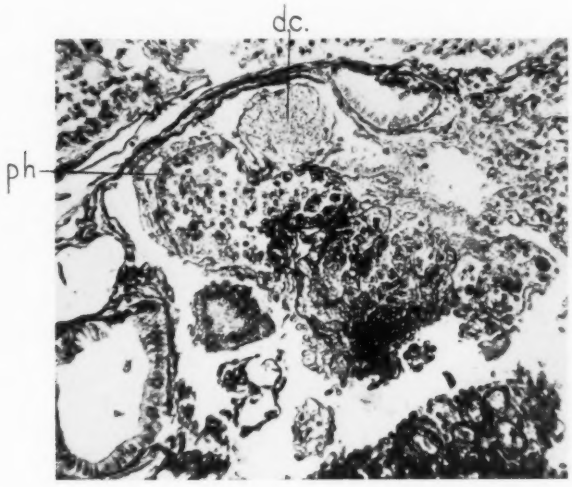

Fig. 21. Glomus of a 36 cm specimen; with phagocytes in the capillaries. ph., phagocyte; d.c., degenerating convolution.

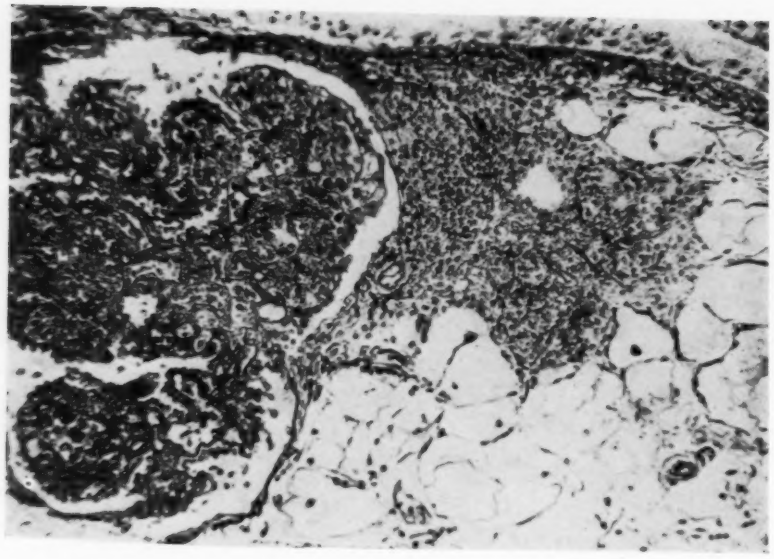

Fig. 22. Formation of a new glomus in a 36 cm specimen. The rudiment of the new glomus is seen to the right. To the left is the functional glomus.

excretory epithelium of such convolutions is poorly developed, the cells being thin and flattened (Fig. 21).

The presence of phagocytes in the convolutions is a problem. I thought at first that they were histiocytes developed from endothelial cells and a token of a degeneration of the convolute, but upon a closer examination of my sections I am now inclined to consider the convolutions which contain phagocytes to be at the beginning of their development. This interpretation is based upon the following facts, gathered from two different kidneys.

A kidney of a 36 cm specimen has a rather small glomus. In this glomus the superficial convolutions in particular are rich in those big phagocytes of which a part lies close to the endothelium of the capillary. Other phagocytes swim freely in the capillary. The excretory epithelium is generally poorly developed on those convolutions which contain many sessile phagocytes, but it is usually well developed in convolutions without such phagocytes. In front of this glomus and in immediate contact with its pericardial pocket, and between this and the pronephric vein lies a big cell-mass (Fig. 22). This mass is surrounded on its internal side by the big fat cells developed in the connective tissue ventrally to the kidney. The cell mass in question is disposed in lobes, of which some are very distinct, others less well differentiated. Between the lobes are strands or lamellae of collagene connective tissue partly connected with the pericardial pocket, partly with the adventitia of the pronephric vein.

VOL. 31 195

The cells contained in the lobes are rather large, polyedrical, with plenty of cytoplasma and a small rounded nucleus. The cytoplasma contains minute granulae and stains palely (in iron-hematoxyline preparations). In the more distinctly outlined lobes, a set of thin cells with flattened, sparse nuclei were found, forming an indistinct delimitation of the lobe against the collagene membranes. A branch of the afferent artery of the glomus penetrates into the rudiment and another artery apparently leaves it. Small, round clear spaces in the cell mass of the rudiment represent small capillaries branching into it.

The fate of the rudiment described just cannot be determined by following its development by steps, as this 36 cm animal is the only one with such a rudiment lying outside the pericardial pocket. A specimen of 34 cm body length, however, gives the clue to this problem. This specimen has a rather big glomus of about the same type as that in the 36 cm specimen. But in this kidney (34 cm specimen) some of the anterior convolutions are solid, filled up by polyedrical cells with small nuclei and their cytoplasma having a fine granulation. A thin layer of very flat cells (endothelium) with flattened nuclei is developed around the cell-mass of the interior of the convolution. The histological picture of such a convolution is just the same as that of a lobe in the rudiment of the 36 cm specimen, so that I do not doubt their identity. In adjacent convolutions the central mass has disintegrated, the central cells becoming free, the peripheral forming an epithelial-like layer inside the thin endothelium. Simultaneously bigger granulae have appeared in the large cells, which are now recognized as being the phagocytes described above.

If this reconstruction of the development of a part of the glomus is correct, the cells contained in the rudiment are phagocytes or prephagocytes.

That the described rudiment is an initial stage in the development of a part of a glomus seems probable from the preceding lines. But there always remains the question of the earlier phases of the development of the rudiment, and my material gives no answer to it. Theoretically it can, however, be interpreted in the following way.

As already mentioned, two arteries are connected with the rudiment, which also contains a set of fine capillaries entering the cell masses of the lobes. When the lobes have been differentiated, the endothelium of the capillaries lies outside the phagocytes. Therefore the whole lobe must belong to the capillary. From this statement it seems to follow that the solid lobes contain an angioblast which differentiates into an endothelium and blood elements of some kind. These blood elements are the phagocytes.

When the rudiment has attained a certain size, it bulges out into the pericardial pocket to form a part of a glomus or form a glomus of its own.

DEGENERATION AND REGENERATION OF PARTS OF THE GLOMUS.

In many head kidneys the collagene membrane or network in some superficial convolutions becomes very thick, through the swelling of the collagene matter, which ultimately seems to dissolve. Such parts of the convolution seem to be thrown off (Fig. 21). In many kidneys convolutions are inflated into big vesicles which may be filled with masses of red blood corpuscles. Such convolutions form a kind of blood sacs. Others are filled to bursting with a coagulate, which in some cases consists of coagulated blood, with a dense mass of fibrine threads.

In these cases it is apparently a question of the degeneration or destruction of a part of the glomus. This must signify that a regeneration must take place in some way. About this regeneration I can only give a few imperfect facts. When the destruction is restricted to a small part of the glomus the regeneration seems to take place from that part of it which is not affected by the degeneration. But if the degeneration affects the entire glomus, the regeneration seems generally to take place from a part of the pericardium at the mouth of the pericardial pocket. In a 40.3 cm specimen the glomus is very voluminous, but its great volume is mainly due to a highly inflated capillary in the centre of the glomus. This capillary contains only a coagulum. Around it the convolute is located in a fairly thin layer with rather wide convolutions, many of which are covered with a sparse layer of flat excretory cells only. In the capillaries are many phagocytes, especially in those convolutions on which the excretory layer is thin (or missing). In the pericardium at the mouth of the pericardial pocket is developed a pad of mesodermal cells clustered around a collagenous mass, connected with the collagenous layer of the pericardium. This collagene mass is covered with the pericardial epithelium, which, however, is here transformed into clublike cells of the same shape as the clublike excretory cells described before (Fig. 23). Besides these cells the pad contains a great many nuclei belonging to cells which may be matrix cells to part of the collagene mass. The mesodermal pad is divided into two parts, of which the anterior part is more advanced in development than the posterior. The latter is more compact than the former, which is penetrated by small capillaries coming from the degenerating convolute. In this part of the pad the excretory cells are so closely packed together that they form a kind of cylindrical epithelium with distally arranged nuclei (Fig. 23). Studying this epithelium closely one will find that the cells are proximally drawn out into long fibres, which proximally enter the collagene nucleus of this part of the glomus. The proximal ends of these threads are transformed into collagene and take part in the formation of the collagenous structures of the glomus. It is often possible to observe that the thread is branched at the end and that the branches are collagenous. A great many of such thread cells do not reach the surface of the pad, but are embedded

VOL. 31 1950

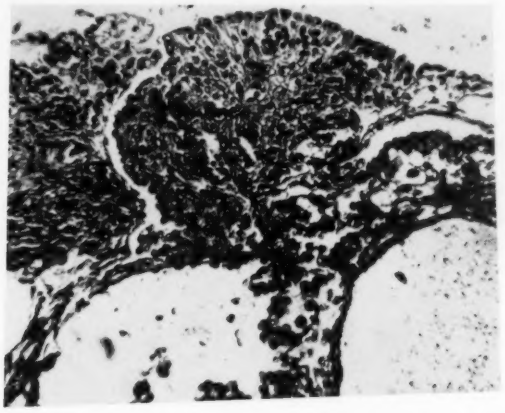

Fig. 23. Regeneration of a part of a glomus in a 36 cm specimen. Club-like young excretory cells in the cell cushion. Below the rudiment are seen two excessively dilated convolutions of the degenerating part of the glomus.

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in the pad at different levels. Such cells will ultimately become the excretory epithelium of the new convolutions as soon as the pad has been definitely penetrated by capillaries.

MESONEPHRIC GLOMERULI.

The mesonephric glomeruli are built up like the pronephric glomus, except that they are shut off from the peritoneal cavity and have an outlet into the mesonephric duct. In these glomeruli also, phagocytes are present in the capillaries of the convolute. The mesonephric phagocytes do not differ at all from the pronephric. They must also be conveyed into the arterial blood stream.

In the mesonephric duct the high epithelial cells are laden with spherical or ovoid bodies of different sizes. These bodies are identical with those that are sometimes delivered from the excretory cells of the glomus, and which, after passing through the pericardial cavity, the tubules of the pronephros and its central mass, are poured out into the pronephric vein. The bodies of the cells of the mesonephric duct are conveyed to the lumen of this duct and are emptied into the exterior. They are solid bodies, so-called "yellow bodies," built up from matter contained in the blood-vessels which spin round the mesonephric duct.

When one of these bodies is about to leave its cell, it becomes surrounded by a clear space. This space, containing the body, bulges out from the surface of the cell like a balloon and then bursts, releasing the body. The inner surface of the epithelium is thus covered with the remnants of such burst balloons.

Fig. 24. Spleen of a middle-sized specimen. The empty spaces are the remains of enormous fat cells, from which the fat is removed. The black nuclei in the centre of the spleen belong to young erythrocytes. They are surrounded by a cell mass, containing the different stages of the development of the blood corpuscles.

VOL. 31 1950

FORMATION OF THE BLOOD CORPUSCLES.

FORMATION OF RED BLOOD CORPUSCLES.

a) In the spleen.

It has long been recognized that the spleen of the hag-fish is represented by "myeloid" tissue, ensheathing the sinusoids in the wall of the intestine. This tissue has, however, not much in common with a real spleen, such as we know it from other vertebrates. It is not consolidated to form a well-defined organ but is distributed discontinuously in the external part of the longitudinal folds of the intestine, between the big fat-cells which fill up these folds. Examining this "myeloid" tissue we find that it is generally composed of small elements. In places, however, bigger cells are present, which, it may be suggested, are initial stages in the formation of blood elements of some description, but I have never found this tissue to contain such developmental stages of blood elements as could lead to red blood corpuscles.

Under certain unknown conditions, however, a real spleen can arise in the myeloid tissue. This is the case in a half-grown specimen, where there are

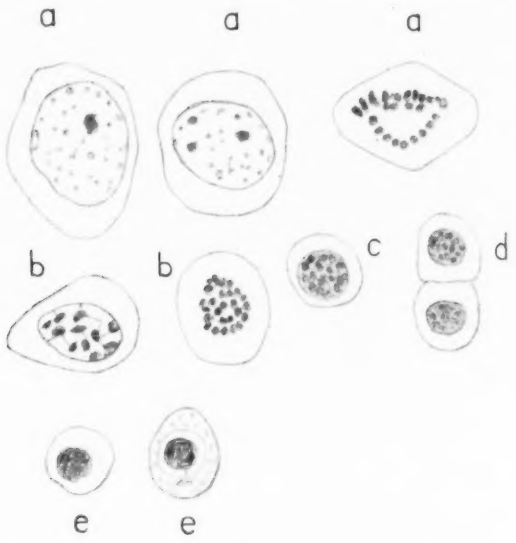


Fig. 25. Developmental stages of the erythrocytes in the spleen. 1 000 ×. a. haemocytoblasts; b. proerythroblasts; c. polychromatic erythroblasts; d. normoblast; e. erythrocytes.

present in the peripheral part of the submucosa of the intestine two well-defined bodies, which apparently represent two distinct parts of a spleen (Fig. 24). The sections show that these parts are built up of a network of collagenous reticular connective tissue, in the meshes of which blood elements are enclosed. These elements form a complete series from the big haemocytoblasts to small "newborn" erythrocytes. The last stages of this development are found in the centre of the spleen, where the network is dissolved.

The blood elements in the spleen are the following:

1) Haemocytoblasts (Fig. 25 a). These are big rounded cells with a diameter of 20—25 μ. Their nucleus is large and rounded, containing one or two nucleoles and a well-developed chromatine network. The cytoplasma is weakly stainable. These cells divide mitotically and give origin to

2) Proerythroblasts (Fig. 25 b), which are cells with a maximum diameter of 17—20 μ. In the cell here illustrated the nucleus contains an indeterminable number of chromosomes. It is preparing for mitotic division. Figure 25 b (second picture) demonstrates a section through the equatorial disc of a dividing proerythroblast. Through this division two

c) Polychromatic erythrocytes (Fig. 25 c), are organized. These are cells with a diameter of 11—12 μ, with round, strongly chromatic nucleus. They divide mitotically and each gives rise to two

d) normoblasts (Fig. 25 d), with a diameter of about 10 μ. The normoblasts

have a round central nucleus, in which the chromosomes are still recognizable. The cytoplasma apparently contains haemoglobin.

e) The young erythrocytes (Fig. 25 e) arise through concentration of the chromosomes into a dark-stained body, in which the chromosomes can no longer be recognized. The young erythrocytes are round and have a diameter of about 9—10 μ. They contain haemoglobin. The nucleus is small and is surrounded by a rather broad border of protoplasma. It is probable that the cells, upon leaving the spleen, change their round shape to an elongated one, viz. attain their definitive form. Newertheless they have to grow somewhat to reach the normal size of an erythrocyte. It must, however, be borne in mind that the size of the "normal" erythrocytes is very variable.

In the spleen of *Myxine*, as described in the preceding pages, no blood elements are formed other than erythrocytes, so that the erythropoietic process there is free from the influence of other haemopoietic processes.

The erythropoiesis here described has very little in common with the conception arrived at when studying the blood in film preparations, arranging the different cells there found in series, proclaiming such series to be developmental series. JORDAN and SPEIDEL (1930) have made an attempt at such an arrangement. The result does not seem to be in accord with the preceding and the following descriptions.

VOL. 31 1950

b) In the liver.

In *Myxine* the formation of blood in the liver generally seems to take place in very young specimens or perhaps already in the embryo. Nevertheless, in a few cases I found that erythropoiesis also occurs in the liver of young animals. There, too, the process is not disturbed by the formation of other blood elements and could be studied accurately.

As is clearly seen from figures 26, a—f, the development of the erythrocytes in the liver coincides fully with that in the spleen. There are haemocytoblasts present. They divide mitotically and form proerythroblasts. These divide and form polychromatic erythroblasts. After division the latter give rise to normoblasts, which are transformed into erythrocytes.

c) In the pronephros.

Formation of red blood corpuscles.

In the pronephros the haemopoietic activity is very complicated because this organ simultaneously forms lymphoid cells and erythrocytes, with the developmental stages of both elements mixed up with one another. Already at the beginning of these investigations it was clear that erythrocytes develop in the pronephros, as during high activity of the organ erythrocytes were found everywhere in the compact body of the central mass. These cells could not have been introduced into the mass, as there are no blood-vessels in it. I also found

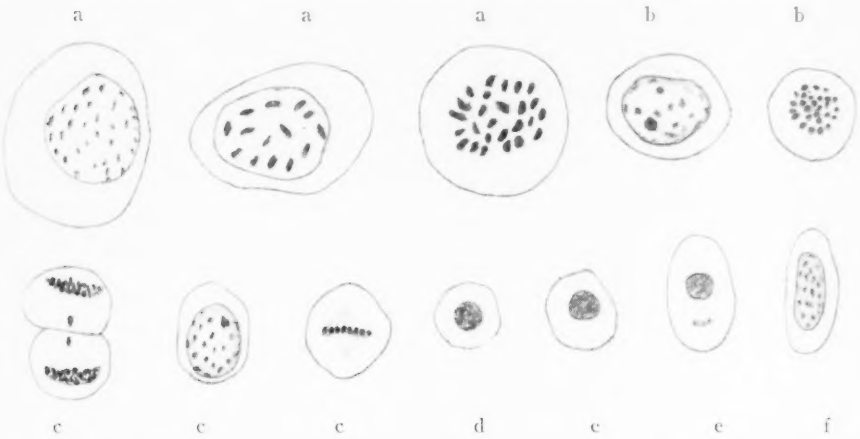


Fig. 26. Developmental stages of the erythrocytes in the liver. 1 000 ×. a. haemocytoblasts; b. proerythroblasts; c. polychromatic erythroblasts; d. normoblast; e. erythrocytes; f. probably a polychromatic erythroblast of oblong form.

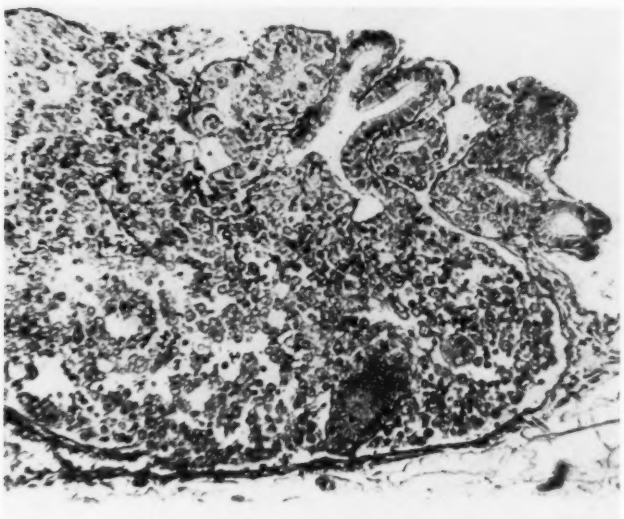


Fig. 27. Section through the pronephros of a 18 cm specimen from which the in fig. 28 figured developmental stages of the blood corpuscles are collected. The central mass is desintegrating.

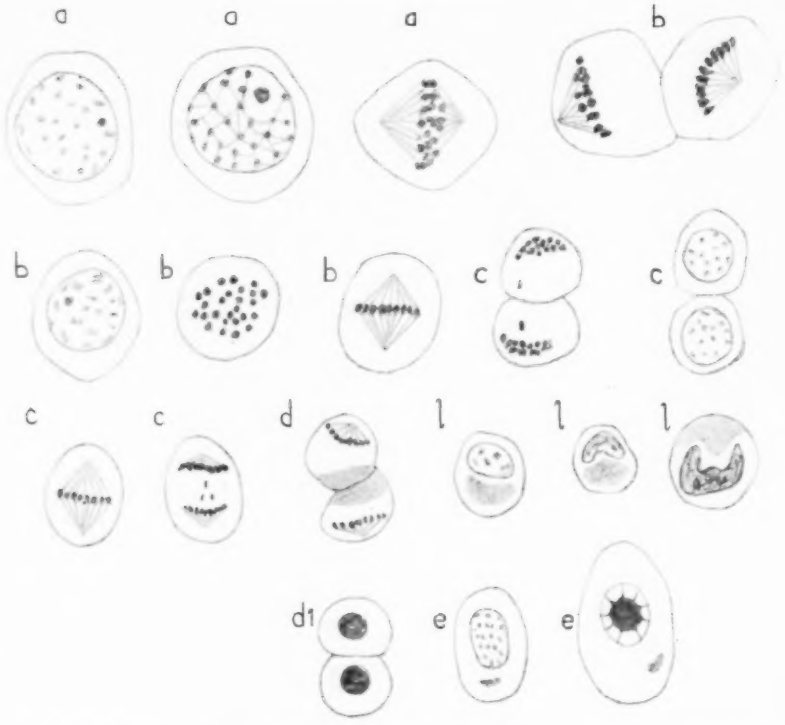


Fig. 28. Developmental stages of the erythrocytes and granulocytes in the pronephros of an 18 cm specimen. a. haemocytoblasts, 1 000 ×; b. proerythroblasts; c. polychromatic erythroblasts; d. normoblasts of the granulocyte series; d_1. of the erythrocyte series; l. granulocytes; e. erythrocytes.

cell divisions in the mass, which could scarcely be anything but the divisions from which the erythroblasts originated. But the analysis of their development could not be carried further so long as I did not know how the erythropoiesis occurred, when no lymphatic elements were present capable of obscuring the picture. In the preceding pages such an unobscured process has been described relating to the spleen and also to the liver. With some knowledge of this "clean" process it is not difficult to make out the erythropoietic process in the pronephros.

In figur 28 the different stages of the process are illustrated. They are gathered from a single pronephros of a specimen of 18 cm body length (Fig. 27).

a) Haemocytoblasts (Fig. 28 a) measuring about 20—22 μ in diameter, with a big round nucleus (15—19 μ), either with only a chromatic network in which chromatic granulae are distributed, or with the chromatine already condensed into short chromosomes. In the latter case the cell is preparing for mitosis.

VOL. 31 1950

b) The result of this division is medium-sized rounded cells of somewhat variable size, 15—19 μ, with a big rounded nucleus (8—12 μ). These cells are proerythroblasts (Fig. 28 b).

c) The proerythroblasts, dividing mitotically, produce the polychromatic erythroblasts (Fig. 28 c), which are cells about 12 μ in diameter. Their nucleus is round, measuring about 8 μ.

d) The dividing of the polychromatic erythroblasts gives rise to the normoblasts (Fig. 28 d), which develop into erythrocytes (Fig. 28 e), without dividing. The nucleus of the normoblasts has, to begin with, a small, dark-stained dense nucleus, which, however, as the cells grow, swells, the chromatine corpuscles becoming visible again. Later, as the normoblasts is transformed into the definite erythrocyte, the chromatine condenses once more. Simultaneously the chromotoid body of the erythrocyte appears.

The development of the erythrocytes of the pronephros thus agrees fully with that of these cells in the spleen and in the liver. The process agrees also with the development of the erythrocytes in the bone marrow of mammals, but with this difference, that in *Myxine* the process ends with the normoblast stage in which the haemoglobin begins to develop. Sometimes, however, the erythrocytes seem to divide in the circulating blood.

Formation of white blood corpuscles.

As already mentioned, the pronephros contains—when in lymphoid activity—lymphoid elements of a different degree of development mixed up with cells forming red blood corpuscles.

It is obvious that the initial stage of the formation of the leucocyte is the same as that of the erythrocyte series, viz. the haemocytoblasts. The further development of the lymphatic elements cannot be separated from that of the erythrocytes until the stage of polychromatic erythroblasts is reached. At that point of the developmental series the development proceeds by two ways: 1) through normoblasts to erythrocytes and 2) from the polychromatic erythroblasts to eosinophilous leucocytes (Fig. 28 c, d). The division of the polychromatic erythroblasts thus gives rise either to erythrocytes or to eosinophilous (fuchsinophilous) leucocytes. In the latter case the immediate result is rounded cells with an excentric rounded nucleus and a rather dense fuchsinophilous (in the sections stained with iron-hematoxylin and acid fuchsin) cytoplasma. The nucleus becomes incisions and ultimately polymorphic (Fig. 28 f). Such cells are common in the central mass of pronephros and in the blood-stream, where the cells—stained with Giemsa stain or May-Grünewald—present an abundance of eosinophilous granules. It seems probable that the development of the leucocytes has passed a series of stages parallel with that of the erythrocytes, where however the different stages could not be differentiated in the two series. It may be that these stages correspond to JORDAN's and SPEIDEL's "large lymphoid

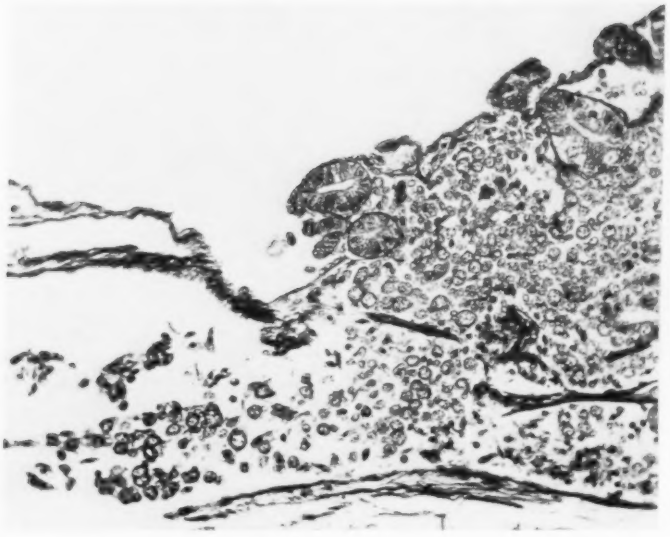


Fig. 29. Pouring out into the pronephric vein of the contents of the central mass of a 10 cm specimen.

VOL. 31 1950

haemoblast" (haemocytoblast), "medium-sized lymphoid haemoblast" (proerythroblast), "small lymphoid haemoblast" (polychromatic erythroblast) and "special granulocytes" and "eosinophils" (eosinophilous leucocytes).

As has been mentioned in preceding sections of this paper, the contents of the central mass of the pronephros is poured out into (Fig. 29) the pronephric vein. When that happens, a large number of blood elements in every stage of development are liberated in the blood, where they either continue the development or are destroyed. It is, however, remarkable how few the mitotic divisions are in the circulating blood. I believe, therefore, that the intravascular development of blood elements is of no great importance for the haemopoiesis.

Phagocytes.

Phagocytes are always present in the head kidney. They are especially numerous after every period of activity, when the central cavity and the central vacuities are filled with remnants of fibre cells, which have to be removed.

In very young kidneys the phagocytes are found especially in the central cavities of the central mass. They arise from fibre cells which are pushed into the cavity or grow into it (Fig. 30). These very young phagocytes keep their connection with their fibres, but later get freed from them (Fig. 31 h). The cytoplasma of such young cells seems finely granulated. As, however, the cell becomes larger, vacuoles appear in which small bodies may be present. Upon

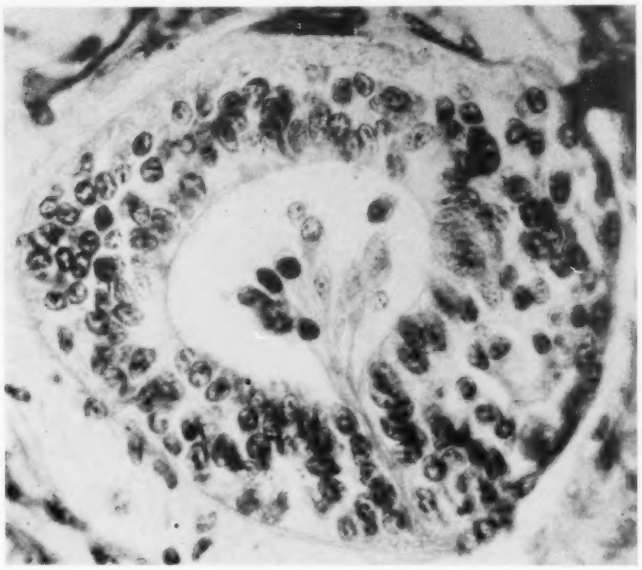


Fig. 30. Formation of phagocytes from fibre cells, wandering through the wall of a tubule in a 40 cm specimen.

injecting living animals in the pericardial cavity with a suspension of carmine one soon finds carmine granules in such vacuoles. When young, the phagocytes display characters of amoeboid cells, later—when laden with phagocyted matter —they have well-defined outlines. Such a round (20 μ diameter) phagocyte is illustrated in fig. 31 a. The nucleus is oblong, and a brown-coloured body is visible in a big vacuole. In fig. 31 b another phagocyte is represented. It is pear-shaped. The nucleus is irregular, oblong. The cell body contains a number of large vacuoles, of which two enclose large elliptical bodies. One of these is a black crystalloid body, which is probably derived from the glomus and has newly been phagocyted by the cell. Such bodies are found free in the central cavity. The other is a brown-coloured body of about the same size as the former. This brown body was apparently a crystalloid like the black one, and represents a first stage in the disintegration of such a body. Fig. 31 c represents a later stage in the disintegration of a crystalloid. Its contents have now been granulous except in a small part in the centrum, which has not yet been transformed to granules. Ultimately phagocytes may be filled with vacuoles containing such granules (Fig. 31 d). When such phagocytes are about to leave the central mass, the granules have dissolved and the cell is now filled up with vacuoles containing a fluid, which colour is lightly blue (Fig. 31 e). The cell may now measure up to 45 μ. But phagocytes filled with granule masses may also be poured into the pronephric vein, where I have found some of them. In

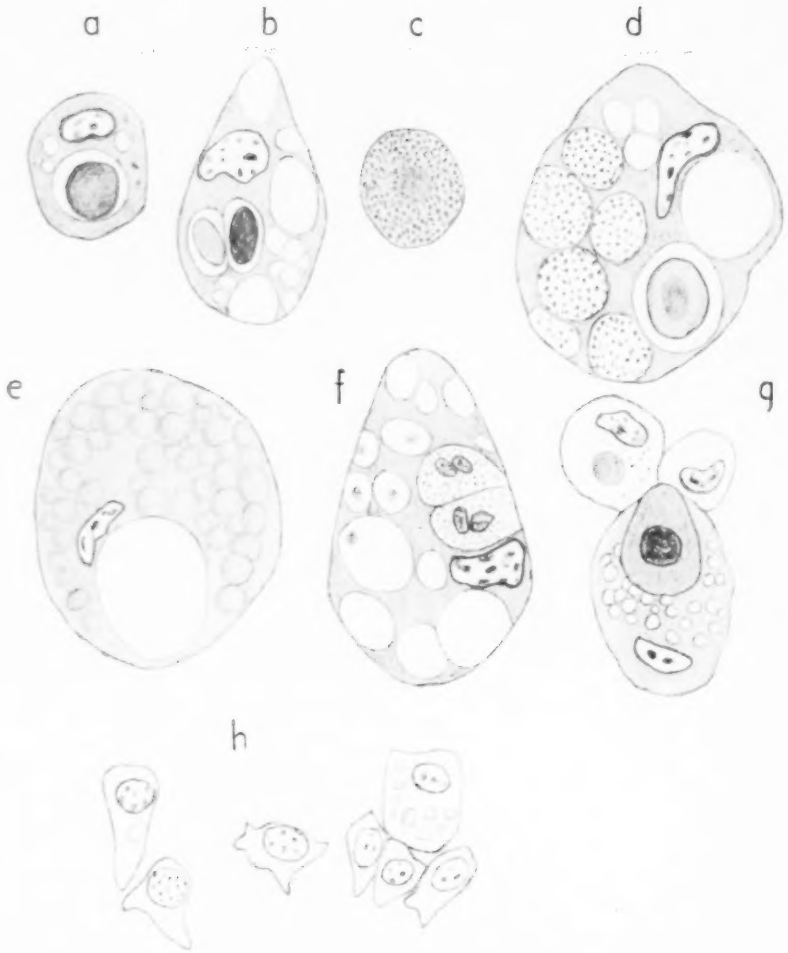


Fig. 31. Phagocytes. 1 000 ×. a, b, d, e, functional stages of big phagocytes from the central mass of a large specimen; c, "yellow" body decomposing; f, phagocyte with two binucleated lymphocytes enclosed; g, three phagocytes destroying a red blood corpuscle; h, young phagocytes from the central cavity.

the circulating blood they seem to be destroyed immediately and I have found remnants of such cells surrounded with their granules or heaps of granules free in the pronephric vein, but not in smears from the circulating blood.

In fig. 31 f I have illustrated a phagocyte, which has devoured two lymphocytes (swelled up in the cytoplasma of the phagocyte) and in fig. 31 g one, that is about to swallow a red blood corpuscle, assisted by two small phagocytes.

Where abundant remnants of fibres are present in the central vacuities it is

VOL. 31 1950

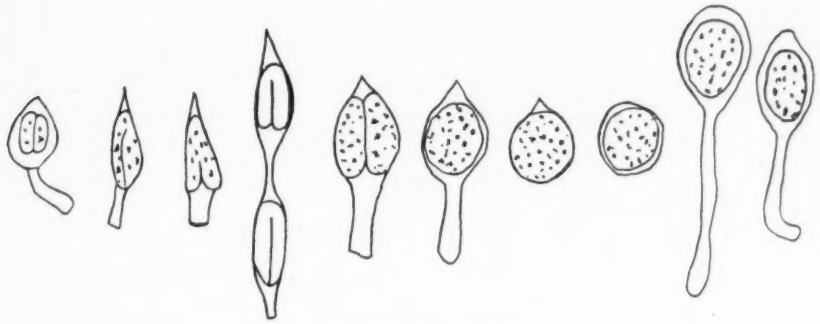


Fig. 32. Spindle cells. 1 000 ×.

obvious that fibre cells, lying upon such fibre remnants, swell up and grow around a group of fibres for instance, which is broken up into granules in the cytoplasma of the cell. Such cells may join others to form big syncytial masses becoming gradually filled with granules. Collagenous trabecles may likewise be attacked and destroyed by big phagocytes.

All these phagocytes are eliminated as soon as they enter the circulating blood, because I could never find in it a single unquestionable phagocyte. It may be, however, that many of the cells degenerating in the blood are phagocytes. In that case they cannot be recognized among all the other degenerating cells of the circulating blood.

The spindle cells. (Fig. 32.)

In blood smears the presence of spindle cells is very conspicuous. Their number is very variable. In some smears they are very numerous, in others their number is less, and in a few cases no such cells were present at all. The cells are very variable. In some cases they form very narrow oblong spindles, attenuated in the "apex" and with a short, sometimes broad, "tail" (Fig. 32). This was the case in a 26 and a 31 cm specimen. The nucleus is long (11—13 μ) and narrow (3—4 μ) mostly cleft along its longer axis or with a longitudinal groove. In other cases (in 12, 15 and 18 cm animals) the "head" of the spindle cell is much bigger, measuring from 14—16 μ with a transversal axis of 8—10 μ. In those thick spindle cells the nucleus fills up the "head", except the apex, and may be longitudinally cleft or remain undivided. The "apex" of the "head" in such big cells is generally short but may be missing altogether. The "tail" is of variable length, generally measuring 10—12 μ, but "tails" 5 μ in length also occur. In some cases the "tail" is missing altogether. In extreme cases I have found spindle cells with rounded heads (without apex) but with a "tail" measuring 20—25 μ (with a head of 13—14 μ). Spindle cells with broad heads and others with narrow heads may be found in the same smear,

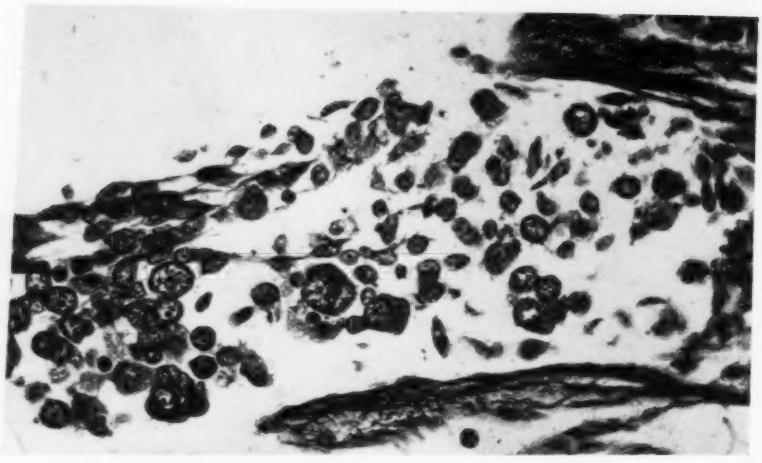


Fig. 33. Formation of spindle cells. Pouring out of the blood elements into the pronephric vein in a 10 cm specimen (10.5). Chains of spindle cells are seen leaving the pronephros.

VOL. 31 1950

and between these two extremes those with intermediate heads. The picture of the supply of spindle cells suggests that the narrow type may develope into the bigger types. Spindle cells without apex and also without tail are also present. These have a rounded nucleus, surrounded by a very thin layer of cytoplasma. Such cells are large lymphocytes.

JORDAN and SPEIDEL believe that such lymphocytes are perhaps cells from which the spindle cells have developed. They have illustrated an evolutional series beginning with such a lymphocyte and ending with a spindle cell with an "apex" as well as a "tail".

Spindle cells are very common in the central mass of the pronephros, and there their development can be studied. As has been described in the general account of the morphology of the pronephros, the blood elements are at a certain stage of their development enclosed in capsules formed by the fibres of fibre cells. When the contents of the capsules are poured out into the pronephric vein the capsules are broken up and capsular cells with the most proximal part of their fibres follow the contents of the capsule out into the vein as small narrow spindle cells. Sometimes the fibre cells (spindle cells) leave the central mass in chains, as seen in the photograph (Fig. 33) of a 10 cm specimen. In the same picture lymphocytes are also seen resulting from the breaking-up of the fibre capsules. The "newborn" spindle cells acquire an "apex" and develop into bigger spindles. Other capsule cells (fibre cells) leave the capsule without any "tail" portion. Such cells are small lymphocytes, which develop into bigger lymphocytes. The small lymphocytes may sometimes have vestiges of the fibre from which they have broken free (Fig. 33, 10 cm animal).

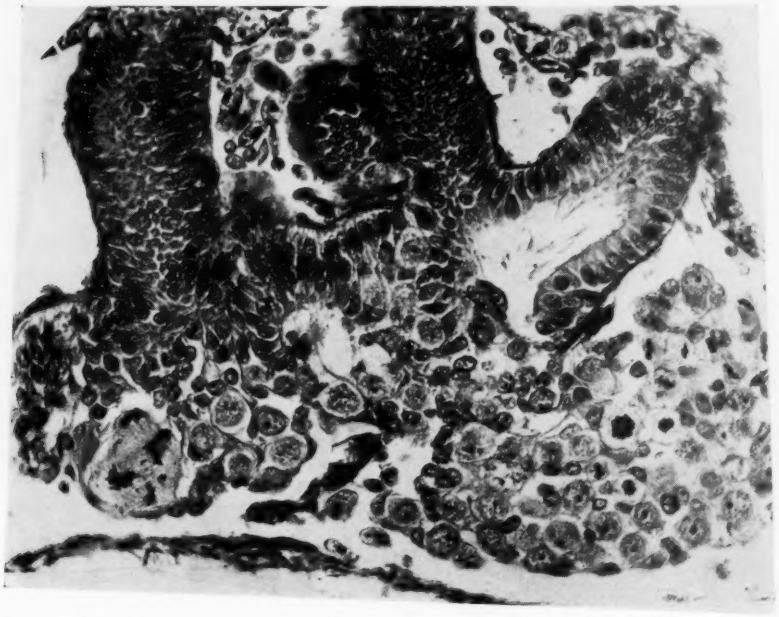


Fig. 34. Central mass of a 13 cm specimen (12.2). Multipolor mitosis of a megacaryocyte. Fibre cell regeneration. Haemocytoblasts.

The nuclei of the fibre cells are variable in form and size (Fig. 32). The fibre cells have mostly elongated nuclei, though rounded nuclei are also very common. Incisions in the nuclei are very common, and longitudinally divided nuclei also occur.

According to these observations it seems possible to establish the theory that spindle cells and lymphocytes in the blood may be sister-cells both originating from the fibre cells of the central mass. If we accept this conclusion the possibility is not excluded that spindle cells and lymphocytes may also be formed in other organs than in the pronephros. So far, however, I have not been able to find out any other organ in which such cells are produced.

The megacaryocytes.

The megacaryocytes are undoubtedly derived from haemocytoblasts. They are very variable, very large cells, mostly with more than one nucleus. The largest cells met with have a diameter of 50 μ, with a great number of nuclei arranged mostly near the surface of the cell body (Fig. 18). Sometimes the nuclei are all of the same size, sometimes the sizes of the nuclei are variable in the same megacaryocyte. The development of megacaryocytes is as follows. A haemocytoblast grows probably rapidly, reaching a diameter of about 30 μ.

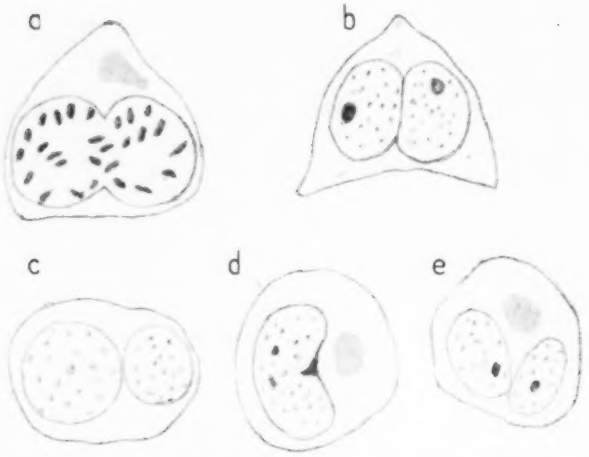


Fig. 35. Megacaryocytes. 1 000 ×. a, d and e, are from the pronephros; b and c from the circulating blood, but also present in the pronephros.

Then the nucleus generally divides mitotically, but the cytoplasma does not divide. This first division of the nucleus is quite normal. Then follows a new division of the nuclei, which may also be normal, but it generally becomes irregular, multipolar (Fig. 34). Polar fibres from one centrosome centre may be attached to 2 or 3 or more equatorial chromosome plates. This condition seems to account for the different sizes of the nuclei in the biggest megacaryocytes. These cells, when examined in sections fixed in Flemming's fluid, contain a large number of big fat drops, mainly distributed superficially (Fig. 18). (See special chapter on fat in pronephros.) The fate of the biggest megacaryocytes is obscure. They are never found in the circulating blood, nor have they been found leaving the pronephros together with the other blood elements. Therefore I believe that they undergo some development in the pronephros. The presence in some cases of groups of blood elements enclosed in a single fibre capsule seems to indicate that at least some of the megacaryocytes end their development with a cytoplasmic division, each nucleus being surrounded by a cytoplasmic mantle of its own. Such a process seems to explain the absence of any larger megacaryocytes in the blood stream.

Binucleated cells (Fig. 35), which are larger than the haemocytoblasts, may be considered to be a kind of megacaryocytes. Such cells get their two nuclei through amitotical division, as is demonstrated in fig. 35. This amitotical division could sometimes be said to be a degenerated mitotic division, as there is a sphere present in front of the incision of the nucleus, which indicates it to divide in two. The fig. 35 a illustrates a pronephric cell in which centrosomes are present at the surface of the cell, but the nucleus divides amitotically. In this

VOL. 31 195

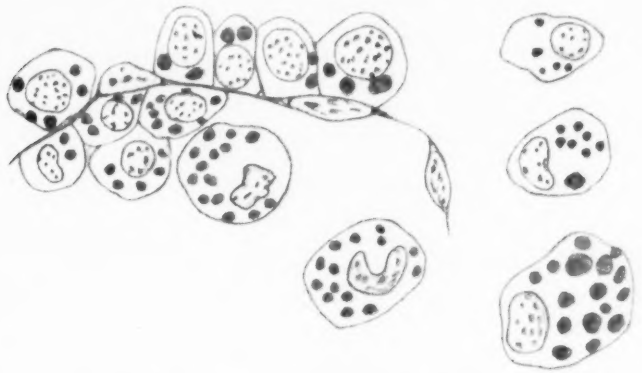


Fig. 36. Heparoblasts with fat-drops. 1 000 ×. From sections of the liver. Outside the endothelial wall of a sinusoid of the liver lie five liver cells, inside it six heparoblasts. Three other heparoblasts are figured at right.

OL.
31
950

case the chromatin of the nucleus is disposed in well-defined chromosomes. It is obvious that this cell had already begun to get arranged for a mitotical division, which, however, did not work. In this case the two nuclei resulting from this division will be of the same size—a conclusion which may perhaps be drawn from the presence in the blood stream of cells of the appearance demonstrated in fig. 35 b. When the nucleus divides perfectly amitotically, one of the daughter-nuclei seems always to be bigger than the other (Fig. 35 c, d, e). Such cells are present in the pronephros as well as in the circulating blood.

Leucocytes with basophil grains and polymorphous nuclei are sometimes met with in the circulating blood. Such leucocytes are rare. Their development could not be traced. Degenerating haemocytoblasts and erythrocytes are common.

In the sinusoids of the liver are found free cells (heparoblasts) of variable size and variable shape of the nucleus (Fig. 36). The size varies from 10 to 23 μ, their nuclei from 5 to 8 μ. The nucleus in small cells is mostly round, in larger irregularly formed, with concave inner and convex outer outline. In livers treated with osmic acid these cells contain a variable amount of fat drops. If we study these cells closely and compare them with the liver cells, it becomes very clear that they are liver cells which have been expelled from their connection with the other liver cells and become free in the sinusoids. The normal liver cells, like the small expelled cells, contain a rounded nucleus and an abundance of fat. The fate of such eliminated liver cells could not be observed, but as there is in the circulating blood not a single cell containing fat, it is obvious that the cells in question, if they leave the liver, must already have used up their fat. But in the circulating blood I could never observe the presence of cells which could correspond to such liver cells without fat deposits. Some big "leucocytes", however, might be such cells. As no degenerating cells

were found in the sinusoids of the liver, the fat-carrying cells must come into the blood-stream or be retained to the liver. The elimination of the liver cells must have some relation to the normal development of the liver, where the destruction and reconstrution of the liver tubes are very normal occurrences.

THE BLOOD OF MYXINE AS IT APPEARS IN BLOOD SMEARS.

The blood of *Myxine glutinosa* has been studied especially by JORDAN and SPEIDEL (1930), and that of *Bdellostoma polytrema* by LAMA SAN MARTIN (1944). JORDAN and SPEIDEL have made their investigations on smears as well as on sections, SAN MARTIN on smears only.

JORDAN and SPEIDEL have described the following blood elements in *Myxine:* erythrocytes, lymphoid haemoblasts (large, medium-sized and small), erythroblasts and normoblasts, special granulocytes, thrombocytes, thromboblasts, eosinophilic granulocytes, monocytes, lymphocytes and spindle cells. Most of these different cell types are present in my smears and, in addition, some others. These others are basophilic granulocytes (leucocytes), phagocytes, megacaryocytes and a kind of minute cells which I do not know how to place. Some dwarfed cells have also been found. In the preceding pages I have described how the blood receives a considerable addition of phagocytes of different sizes. Such cells have not been recorded in JORDAN and SPEIDEL's account.

My description of the development of the different cells of the blood does not coincide at all with the evolutional series figured by JORDAN and SPEIDEL. I admit, however, that using the smears as my only guide, I should have established series similar to those of these authors.

I shall now proceed to make some remarks about some of the blood elements contained in the smears, stained according to May-Grünewald, Giemsa, Pappenheim, Wright and others.

ERYTHROCYTES.

As is already well known, the red blood corpuscles in *Myxine* are generally oval discs of a normal length of 25—30 μ. However, corpuscles measuring from 15—35 μ are also present. The smallest and the largest, however, are not very common. In sections of a 42 mm specimen the size of the red blood corpuscles varied between 15 and 17.5 μ and in a 9.6 cm specimen they were about 20 μ. In section, the corpuscles show an elevation, where the nucleus is located, and a concavity around the nucleus (JORDAN and SPEIDEL). The nucleus generally lies in the middle of the corpuscle, but it may often also be located excentrically. This is especially so when—as is not infrequently the case—the corpuscle is extended acutely at one of its ends. Generally the nucleus is round or some-

what oblong, measuring 6.25—7.5 μ, but sometimes corpuscles are found with big nuclei, 12.5 μ, and cells with a long nucleus, which is frequently laced-in at the middle. Nor are binucleated corpuscles rare. The chromatic substances are generally densely crowded, though nuclei also occur in which the chromatin bodies are more scattered.

In common film preparations the cell body appears to be homogeneous: in bensidin preparations light brownish, in Gimsa or May-Grünewald preparations either uncoloured or light blue or pale rose, depending upon the duration of the staining or the washing of the film. But even in such preparations indications of a structure are visible. Frequently (if not always) a condensation of the cytoplasma is observable a short distance from the nucleus, about midway between it and the end of the cell. This condensation will be called *the chromatoid body*. In erythroblasts (normoblasts) JORDAN and SPEIDEL mentioned a "juxtanuclear spheroidal body", but not in the erythrocytes. Small rounded vacuoles are also seen, irregularly scattered in the cell body. In smears overstained with methylene blue strongly blue-coloured granulae are visible in a more lightly coloured ground substance. These granulae are either round or irregularly formed, somewhat angularly outlined. In preparations overstained with eosin similar but red granulae are seen. I consider these granulae to be artefacts caused by the staining fluid's being retained in the cytoplasma. But even if the coloured bodies should be artefacts, they tell us something about the structure of the cytoplasma. The nature of this structure may be studied in sections preserved in Flemming's fluid or in the bichromatic-formalin-acetic acid fluid of Smith and stained with iron-hematoxyline and fuchsin S. In the Flemming preparations at least three different structures are seen: 1) the very darkly stained chromatoid body, 2) a large number of small dark bodies, each enclosed in a clear space, and 3) a fuchsinophilous fine alveolar plasmatic network. In the Flemming preparations this network is not easily discernible, but in the Smith sections it is very clearly defined.

The chromatoid body seems always to be present in *Myxine*. It also occurs in *Heptatretus* (Bdellostoma) *hexetrema*, but was not found in *Bdellostoma polytrema* (SAN MARTIN), where, however, it may be present. In young specimens of *Myxine* it stains perfectly as the chromatin of the nucleus (iron-haematoxyline), but in older specimens the chromatin of the nucleus retains the dark colour longer than the chromatoid body. This may signify a certain chemical change in the body. The chromatoid body has generally a somewhat quadrangular shape, but it may also be a thin disc or be rodlike, bent like a horseshoe, with the concavity directed towards the nucleus. The body may lie immediately at the nucleus or be located more distantly from it, though generally it is nearer the nucleus than the "hind" margin of the blood corpuscle. Sometimes the body is imperfectly divided into two or there may be two bodies, one on each side of the nucleus.

The nature of the darkly stained bodies mentioned above is unknown. In preparations fixed with Flemming's fluid they are dark when stained with iron-hematoxyline. As no dark bodies are found in smears fixed in Flemming's fluid, the possibility that they are fat drops seems to be excluded.

In sections fixed in Smith's fluid and stained with iron-hematoxyline-fuchsin S, the fuchsinophilous network is very conspicuous. A ring of alveoles is present around the nucleus. The ring leaves a clear space around the nucleus, sometimes seen in smears. Outside this ring the alveoles are much smaller. Their walls contain minute granules. The chromatoid body is also surrounded by a ring of larger alveoles. When the chromatoid body is situated near the nucleus, the two structures have some of the large alveoles in common. The alveoles are empty, but must have contained the dark bodies of the sections fixed in Flemming's fluid, or the blue or red bodies in the smears, described before. There are no circular fibres at the margin of the blood corpuscles, as is the case in Dipnoi.

VOL
31
195

HAEMOCYTOBLASTS.

The haemocytoblasts of the blood are variable in form and size (Fig. 37 a). They always have a big nucleus, staining strongly violet. The cytoplasma always stains blue in May-Grünewald or Giemsa stains. The haemocytoblasts are either spherical or more or less ovoid. The round type generally has a diameter of 20—25 μ. The ovoid cells may have a long axis of 22—35 μ and a short axis of 11—15 μ. The round cells were never found with the chromatin of the nuclei disposed in chromosomes. It was always densely distributed as grains in the nucleus. The ovoid type, on the contrary, often has the chromatin of the nucleus in the form of chromosomes, and such cells are also found dividing mitotically in the blood. However, the number of mitoses in the smears is very small. Therefore the formation of blood corpuscles in the circulating blood plays but an insignificant rôle.

Whereas the haemocytoblasts in the central mass of the pronephros are still all rounded bodies, in the circulating blood a great many of them are more or less elongated. In the pronephros the round divide with lively activity, but in the blood only elongated corpuscles are seen dividing. This condition can scarcely be given any other interpretation than that some of the haemocytoblasts become elongated when set free in the blood.

PROERYTHROBLASTS.

In the circulating blood are many proerythroblasts (the medium-sized lymphoid haemoblasts of JORDAN and SPEIDEL) (Fig. 37 b). They are generally somewhat elongated with a longer axis of 18—20—30 μ and a shorter

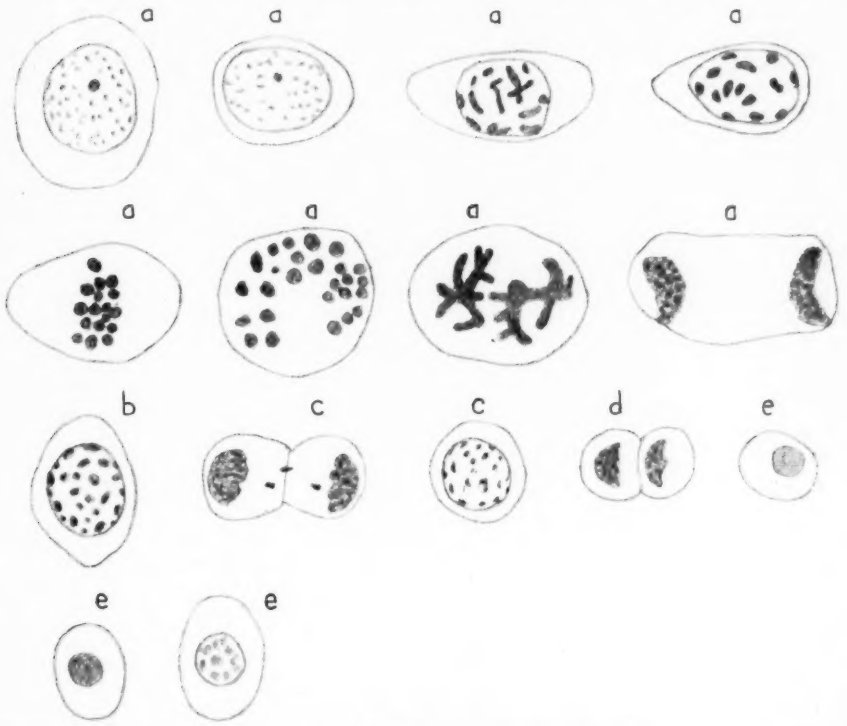


Fig. 37. Developmental stages of red blood corpuscles collected from smears. 1 000 ×. a. haemocytoblasts; b. proerythroblast; c. polychromatic esythroblasts; d. normoblasts; e. young erythrocytes.

of 8—10—14 μ. The nucleus is generally about 12 μ in diameter. Their cytoplasma is coloured blue in different shades. The chromatin is granulated; when the cell is preparing for division, disposed in chromosomes. The division is a typical mitosis, but in the diaster stage a few chromosomes do not follow the rest of them to the spindle pole but are left behind near the equator of the dividing cell. As I have seen such a picture repeatedly, I think it must be a somewhat regular phenomenon. It seems possible that these single chromosomes are the rudiment of the chromatoid body of the mature erythrocyte.

POLYCHROMATIC ERYTHROBLASTS.

From the division of the proerythroblasts result two "small lymphoid haemoblasts" or polychromatic erythroblasts (Fig. 37 c). These are rounded cells with a diameter of about 15—18 μ and with a nucleus diameter of 8—10 μ. The cytoplasma is pale blue, the chromatin violet, when resting granular, when preparing for division including chromosomes. The division is typically mitotic.

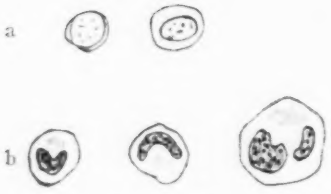


Fig. 38. Lymphocytes (a) and eosinophils (b). 1 000 ×.

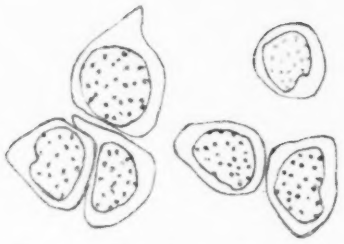


Fig. 39. "Trombocytes". 1 000 ×.

NORMOBLASTS.

Through the division of the polychromatic cells there arise normoblasts (Fig. 37 d), which at the outset are the smallest cells of the erythropoietic series. In this stage haemoglobin seems to develop, and simultaneously the cells swell considerably (Fig. 37 e), attaining at length the size of the mature erythrocytes.

LEUCOCYTES OR GRANULOCYTES.

In the circulating blood there are many granulocytes. They never seem to arise in the blood but, as far as I know, they originate either in the head kidney or in the folds of the intestine, as demonstrated by JORDAN and SPEIDEL. I never found a single unquestionable case where a granulocyte has developed through division or transforming of any other cell element of the blood. Very common in the blood are the eosinophilous (or fuchsinophilous) granulocytes. Basophilous granulocytes are also present but in inconsiderable numbers. Neutrophilous (special) granulocytes have been described by JORDAN and SPEIDEL, and I have also seen such cells, but I am not sure that they are different from the eosinophilous cells, as they assume a slight rose shade in the Giemse or Wright smears. JORDAN and SPEIDEL have found them developing in the splenic cords of the intestinal submucosa.

The eosinophilous granulocytes (leucocytes) are cells of different sizes (Fig. 38 b). The youngest (smallest) measure about 7 μ, the biggest may attain a diameter of 18—20 μ. As to size, every transition between the smallest and the biggest may be present. It is certain that these cells grow during their vascular life. Their nucleus is more or less irregular, sometimes forming a short or long bent band, sometimes being broken up into two pieces. The granules are very fine, almost dustlike, but cells have also been found with coarser granules. Granulocytes having their origin in the pronephros and derived from the hemocytoblasts cannot be separated from those derived from the splenic cord of the intestine. The eosinophils grow considerably in the circulating blood.

The basophilic granulocytes, of which I have found only very insignificant numbers, have generally a lobed nucleus and coarse blue granules in the cytoplasma. Their origin is unknown.

MONOCYTES.

JORDAN and SPEIDEL have figured two monocytes, which they derive with some doubt from "small lymphoid hemoblasts". One of these monocytes may be a special granulocyte or belong to the lymphocyte series (see below). The other is a rather big cell containing vacuoles and dark inclusions. This cell is probably a phagocyte. I have found no cells which can unquestionably be referred to as monocytes.

LYMPHOCYTES.

These are generally small (5—6 μ) with a small amount of cytoplasma (Fig. 38 a). In another part of this paper I have described the origin of a considerable amount of them from fibre cells in the head kidney. But lymphocytes may probably be derived from other sources. In the circulating blood the lymphocytes grow considerably (to 10—12 μ) without changing their general appearance. Their nucleus is generally round, but nuclei with incisions and also cells with two nuclei are common.

THROMBOCYTES.

JORDAN and SPEIDEL have described and depictured rather large irregularly shaped cells, with variously shaped nucleus and pseudopodiumlike processes of the cell body. These authors found a few thromboblasts from which they derived the thrombocytes. I have found many cells which I consider to correspond to the thrombocytes described by JORDAN and SPEIDEL. They were clumped together in small groups and were of different sizes. The smallest of them do not differ at all from large lymphocytes and may easily be derived from such cells (Fig. 39). In my smears the thrombocytes have a homogeneous pale-blue cytoplasma such as has been described in some thrombocytes (JORDAN and SPEIDEL).

SPINDLE CELLS.

The spindle cells are very common in the circulating blood (Fig. 32), but their numbers are very variable. In some specimens they are few, in others very numerous. They certainly do not develop in the blood. In preceding pages I have described their origin from fibre cells in the head kidney. When young

they have a long (about 10 μ), often grooved or bipartite narrow (3—4 μ) nucleus, a short tip at the anterior end and a "tail" of variable length at the posterior end. The young spindle cells grow in the circulating blood. Their nucleus becomes oval, 13—14 μ long with a width of about 10 μ. Their "tails" are broadened. Sometimes the tail is missing, sometimes it is very long, for instance in a 40 μ cell the tail is 26 μ long. Sometimes young spindle cells may be united to form short chains.

PHAGOCYTES.

In the blood smears phagocytes are rare, if they ever occur. This is a very puzzling fact, as we know that masses of phagocytes are poured out in the pronephric vein and also in the efferent arteries of the glomeruli. The explanation must be that they are immediately destroyed in the circulating blood. A few cells with small nucleus, plenty of cytoplasma with inclusions have been interpreted as not yet destroyed phagocytes. For possible remains of phagocytes in the blood see further on.

VOL.
31
195

HEPAROBLASTS.

Such cells, described on p. 285 (Fig. 36), are not present in my smears, but they occur, at least temporarily, in the sinusoids of the liver.

MEGACARYOCYTES.

Binucleated big cells (25—30 μ) have been interpreted as small megacaryocytes (Fi. 35). The division of their nucleus is either a reduced mitosis or a kind of amitosis. In the first case chromosomes are formed in the nucleus but no spindle, in the second the nucleus is constricted in the middle and two nuclei are formed. Generally one of these is larger than the other. As evidence of a degenerated mitosis in this case also an archoplasmatic body is present outside the incision of the nucleus. These megacaryocytes are apparently descendants of hemocytoblasts. Polynucleated megacaryocytes do not occur in the circulating blood.

MICROCYTES.

In a few cases (in a 26 cm specimen) I have found groups of minute bodies, here called microcytes (Fig. 40). These bodies are more or less triangular in shape with a nucleus-like basal part and a light tip opposite the "nucleus". The bodies' measure 3—4 μ. The bodies are always aggregated and make the appearance of a larger cell that had become fractioned and every fragment had

Fig. 40. Microcytes. 1 000 ×.

acquired its part of the chromosomes of the original cell. Other dwarf-like elements are present in the same smear. In two cases a chromosome-like body is surrounded by a clear homogeneous cytoplasma, which may in the larger of these "cells" (10 μ) contain haemoglobin (Fig. 40). A third such cell measuring 8 μ has a very small real nucleus, with membrane and granular chromatin (Fig. 40).

CELLS DEGENERATING IN THE CIRCULATING BLOOD.

Degenerating cells have been described in the blood of *Myxine* (JORDAN and SPEIDEL) as well as of *Bdellostoma* (LAMA SAN MARTIN). These authors describe the degeneration of red blood corpuscles. Their descriptions do not agree closely with one another, and I have difficulty in comparing their results with my own. Perhaps the degeneration is not always effected in the same way. Besides, many different cells degenerate in the blood, although the authors have not laid stress upon that fact. It seems possible, therefore, that stages of degeneration of different cell types have been thrown together.

ERYTHROCYTES.

The descriptions of JORDAN and SPEIDEL and of LAMA SAN MARTIN of the degeneration of the red blood corpuscles do not agree well with my observations, according to which the process is as follows.

At the first stage of the degeneration the cytoplasm of the red blood corpuscle is uncoloured but has very discrete outlines. The nucleus is coloured dark violet with easily discernible cromosome-like chromatin bodies. The nucleus emits short rows of chromatic granulae, radiating from the nuclear membrane and partly scattering in the cell body (Fig. 41). These granules are coloured reddish-violet. In a following stage the chromatin of the nucleus is disposed as in the preceding stage, but now is coloured reddish-violet, with not very well marked chromatin bodies. The nucleolus—present already in the preceding stage but hidden by the dark chromosomes—is now quite discernable, blue in colour. Reddish granulae radiate from the nucleus out into the cell body, which is always well delimited (Fig. 41). At length the cell body is filled up by such granulae. Now its outlines have disappeared, but the granulae still remain inside an area corresponding to the outlines of the corpuscle. The

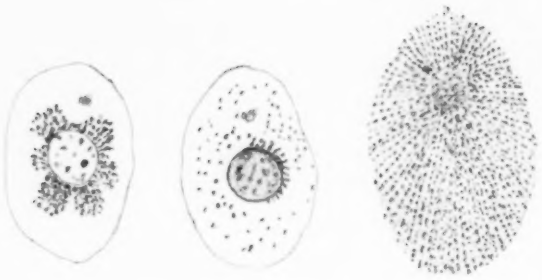


Fig. 41. Degenerating erythrocytes. 1 000 ×.

granulae must thus be contained in a fluid corresponding to the cytoplasma of the cell. During the last part of the degenerative process the cell has swelled considerably. The nucleus is always discernible but has lost its chromatin and all structure, and is only a condensed part in the granular mass from which the granulae radiate (Fig. 41).

VOL. 31 1950

HAEMOCYTOBLASTS, PROERYTHROBLASTS AND POLYCHROMATIC ERYTHROBLASTS.

Haemocytoblasts also degenerate in the circulating blood. The first sign of degeneration consists in the nuclear chromatin assuming a reddish colour. The nucleolus has become blue, and red granulae have gathered along the cell membrane (Fig. 42). In a following stage the nuclear membrane has disappeared on one side of the nucleus and reddish chromatin granulae stream out into the cytoplasma, filling it up completely (Fig. 42). More and more of the nuclear membrane disappears, and finally the entire haemocytoblast is transformed into a granular mass, which at last loses its outlines.

The degeneration of proerythroblasts and polychromatic erythroblasts is similar to that of the haemocytoblasts on a minor scale.

Another kind of degeneration occurs when the haemocytoblasts prepare for mitosis. A normal mitotic figure is not always formed, but the chromosomes are scattered irregularly in the cytoplasma. The chromosomes, moreover, are bigger than in sections (Fig. 37 a). Sometimes they may join on to branched structures (Fig. 37 a). It is difficult to imagine that a normal mitosis can follow upon a monaster stage of this kind. It is possible that such dividing cells may fragmentate and fragments containing one or a few chromosomes may arise in a small amount of cytoplasma. The microcytes may possibly have arisen in that manner.

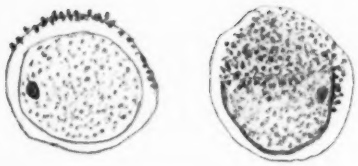


Fig. 42 Degenerating haemocytoblasts. 1 000 ×.

Fig. 43 Degenerating lymphocytes. 1 000 ×.

LYMPHOCYTES AND SPINDLE CELLS.

In the circulating blood lymphocytes as well as spindle cells grow considerably, the former from 8 to about 14—15 μ, the latter from 9 to 18 μ (length of the head). The growing process seems to end in degeneration.

The process of degeneration of a lymphocyte is as follows (Fig. 43). The cells swell even more. The chromatin of the nucleus, earlier disposed in easily discernible granules, now no longer has any distinct granulation but the nucleus is vacuolated, coloured reddish violet. It is surrounded by reddish violet granulae, which seem to emerge from the nucleus in the pale blue cytoplasma. These granulae successively fill up the cell body. But simultaneously a thin uncoloured zone has formed around the vacuolated nucleus. This zone is traversed by short plasmatic fibres. The zone thus seems to be formed by a ring of small alveoles. The vacuolated nucleus may now shrink or it can keep its round form. Th uncoloured zone becomes narrower and the reddish grains in the cytoplasma fill it up and in addition pass out from the cell, which by now has lost its cell membrane.

This process of degeneration corresponds closely to that described by earlier authors as taking place in degenerating red blood corpuscles.

The spindle cells degenerate in the same way as the lymphocytes.

Fig. 44. Degenerating eosinophils. 1 000 ×.

GRANULOCYTES.

The eosinophilous granulocytes grow in the circulating blood from 9 to 16 (18) μ in diameter. The smallest types contain very fine eosinophilous granulae (Fig. 38). As such cells attain their larger sizes, the cytoplasma becomes altered (Fig. 44), there arises a zone, more or less free of granulae, around the differently shaped nucleus. This zone is stained bluish. The granulae have become coarse, stained reddish violet. They are densely crowded in the cytoplasma outside the blue zone. In this zone such granulae are sometimes seen quite distinctly against the blue background. The blue zone broadens, and simultaneously the amount of granules decreases, granulae piercing the cell membrane and becoming free in the blood fluid. Ultimately the outline of the cell becomes uneven, the nucleus vacuolated and the cytoplasma uncoloured, all granulae being dissolved or set free in the blood. The fate of these empty cells could not be determined, but probably they are dissolved in the blood fluid.

OTHER BLOOD ELEMENTS.

Degenerative processes in the other blood elements have not been observed with any certainty in my smears.

THE PRONEPHROI OF SPECIMENS OF DIFFERENT SIZES.

An investigation into about 1 000 serial sections of head kidneys of *Myxine* specimens of different sizes (from 9.3 to 46 cm) has disclosed that there are two extreme types present, viz. one type in which the fibre tissue dominates in the central mass and one in which large rounded cells—blood elements—give the mass its essential character. In the first case the central mass mostly occupies only a minor part of the pronephric vein, in the second it may fill up the vein almost completely, so that there is but a narrow vein space surrounding the mass. Between these two extreme types are transitions, indicating that they form stages in an evolutionary or functional process. These types are most typical in young animals (9.6—13 cm). In older animals the types are not always possible to separate, as one part of the central mass may belong to the

VOL. 31 1950

one type, another to the other, or as the blood elements may be very few. Moreover, the fibrous type can assume different aspects, depending upon destruction and regeneration taking place in different parts of the central mass. In older animals degenerative processes and phagocytosis occurring regularly in the central mass are responsible for many peculiarities of the aspect of the central mass.

The two types of head kidneys will be called: the fibrous and the cellular or lymphoid types respectively.

The head kidney of a specimen 4.3 cm in body length.

As this specimen was very badly preserved it was not possible to obtain a detailed picture of its organization. The tubules are few (about 14—16). Basally they are connected with the central mass, which forms a single mass throughout the whole kidney. In this mass two kind of cells are present, one with rather large rounded and another with smaller, somewhat oblong, nuclei. Threadlike structures are also present. Probably the latter belong to the smaller cells, which may be fibre cells. This kidney belongs to the lymphoid type.

The head kidney of a 9.3 and a 9.8 cm specimen.

The 9.3 cm specimen has a small pronephros with rather few tubules (15—20), some of which are branches of others. The tubules, some of which are rather long, unbranched, others branched but shorter, end basally in a somewhat widened spherical thick-walled cavity. This cavity opens basally into a system of vacuities with many phagocytes. The hollows are surrounded by fibre cells, the fibres of which are fastened at the bases of the tubuli. The fibres form a kind of reticulum, in the mashes of which rounded cells are enclosed. As no younger specimens are available it is not possible to know the stage of functional activity of this kidney. I suppose, however, that it is at the very beginning of a lymphoid stage. Haemocytoblasts were not found. The cells enclosed in the meshes of the central mass are young phagocytes and lymphocytes.

In a 9.8 cm specimen the head kidney is generally similar to that of the 9.3 cm specimen, but the enclosed round cells are very few in number and the fibrous tissue much more strongly developed. In the basal part of the tubules are some phagocytes. Such cells are also present in the vacuities of the central mass. It seems to me that this kidney is somewhat nearer to lymphoid activity than the preceding one, seeing that big cells are present at the basal end of one of the tubules.

The head kidney of a 9.6 cm specimen.

The pronephros of the specimen has 25—30 tubules, of which 19 are directly connected with the undivided central mass. Each of these main tubules open

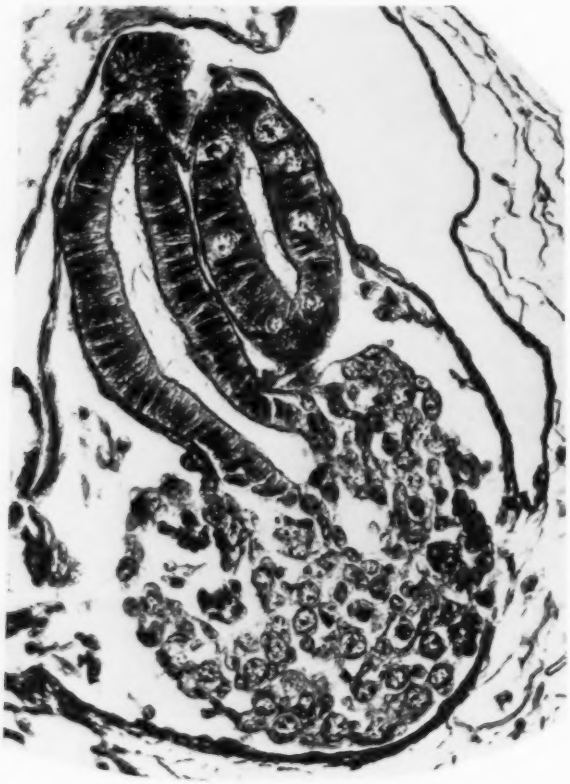


Fig. 45. Pronephros of a 9.6 cm specimen. With haemocytoblasts and proerythroblasts in the central mass. With formative cells (haemocytoblasts) in the right tubule.

into a central cavity of its own. The other tubuli have branched out from the one just mentioned. The tubules are arranged in a dorsolateral and a lateral series. The main tubules are long, generally consisting of typical columnar cells; but in a few of them the tubules are big rounded cells (haemocytoblasts)[1] intermingled with the columnar cells. The haemocytoblasts have a big round nucleus containing a great many chromatic granulae and one or two larger nucleoles. The amount of cytoplasma is not very great. It is nearly uncoloured in the sections stained with iron-haematoxylin. In the actual series no inclusions are seen in the cytoplasma, but in similar sections of somewhat older specimens (10 cm body length) treated with osmic acid similar cells contain fat granulae.

[1] In the following I call this class of cells haemocytoblasts, though I am well aware that many of them belong to the leucoblast—myelocyte—leucocyte series. But I could not differentiate the erythrocyte series from the myelocyte series until the last stage of development.

VOL. 31 1950

Most of the tubuli have a funnel-shaped inner end sunk somewhat in the central mass, or they end with a somewhat widened part dipping into the mass (Fig. 45). The central cavity contains some phagocytes, originating from fibre cells growing out into the cavity. They sometimes were observed with their fibres penetrating the columnar cell layer of the funnel. It is sometimes clearly seen that the central cavity communicates with vacuities in the central mass, there forming part of spongious spaces and meshes. The outline of the central mass is formed by fibre elements packed together at the surface of the mass. There is no endothelium covering the mass.

In the meshes of the central mass free elements occur. They are:

1) large mononuclear cells: haemocytoblasts or leucoblasts. 21—25 in diameter with a nucleus of 14—17.5. In the actual kidney these cells are not numerous. They are located near the surface of the central mass or round the funnels of the tubules.

2) medium-sized mononuclear cells: proerythrocytes or myelocytes. Cell-diameter 14—17.5, nuclear diameter about 10—10.5. They arise through mitotic division of the large mononuclear cells and are located everywhere in the central mass, but in greater numbers at its surface, where some of them are seen entering the pronephric vein.

3) Small mononuclear cells: polychromatic erythroblasts or small myelocytes, arising through mitotic division of the medium-sized cells.

4) Megacaryocytes may be considered to be overfed mononuclears. They are not numerous. Some of them are provided with a number of nuclei, arising either through mitotic division (multipolar) or through amitotic division.

5) Lymphocytes (and spindle cells) are common in the meshes of the fibrous tissue.

6) Phagocytes are present, especially in the central cavity.

Head kidneys of 10 cm specimens.

In 15 pronephroi of 10 cm animals twelve (80 %) were haemopoietically active.

Already at a first glance at the 12 lymphoid pronephroi of the 10 cm animals it is clearly understood that the lymphoid activity is differently advanced in the different kidneys. The differences are really so great that it seems impossible to arrange with any certainty these pronephroi in a series foreshadowing stages in a single evolutionary process. This seems to be due to the fact that the pronephroi are never identical when the process begins, and that the degree of intensity of the lymphoid process differs in different animals perhaps answering to different needs of blood elements in the body system. As blood elements arise not only in the pronephros there may perhaps be a certain correlation between the haemopoietic organs, so that one acts as a deputy for the other and *vice versa*. Nevertheless there must be a certain evolutionary process for

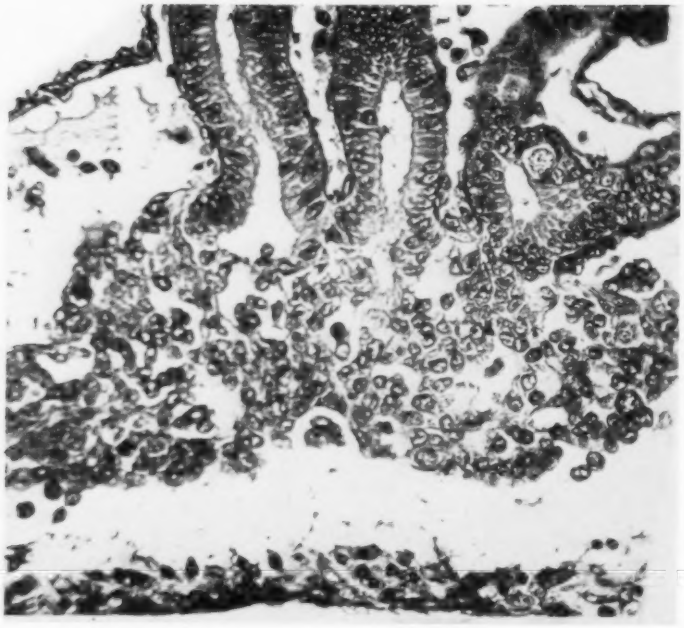


Fig. 46. Pronephros of a 10 cm specimen (10.1). Essentially fibrous type; long tubules.

VOL. 31 1950

each head-kidney, but as one given kidney can only be studied once, its future development must be obscure, especially if the intensity of the haemopoietic activity should suddenly increase or decrease. In the following description I shall, however, if possible, try to analyse the different kidneys according to the preceding principles.

Head kidney 10.1 (Fig. 46). This is a rather small, slender head kidney, about 1.5 mm long. The tubules are not very numerous. They are rather long, thick-walled, with a well-developed mouth-piece. A regular columnar epithelium is present, especially in the apical half of the tubules. In the posterior tubules regular columnar epithelium in developed throughout the entire tubules. In the medium and anterior part the mid-portion and the basal part of the tubules contains many large formative cells (future haemocytoblasts). The central mass is dense, rather slender, pierced only by a small number of slender trabecles. The central cavities are but very slightly pronounced, consisting of little more than the somewhat widened basal portion of the tubules. In these cavities groups of cells project from the central mass into the cavity. These cells have an irregularly formed cell body with a rounded or somewhat oblong nucleus. They seem partly to be suspended by the cilia of the basal part of the tubule (infundibulum). They are apparently phagocytes. The fibrous tissue of

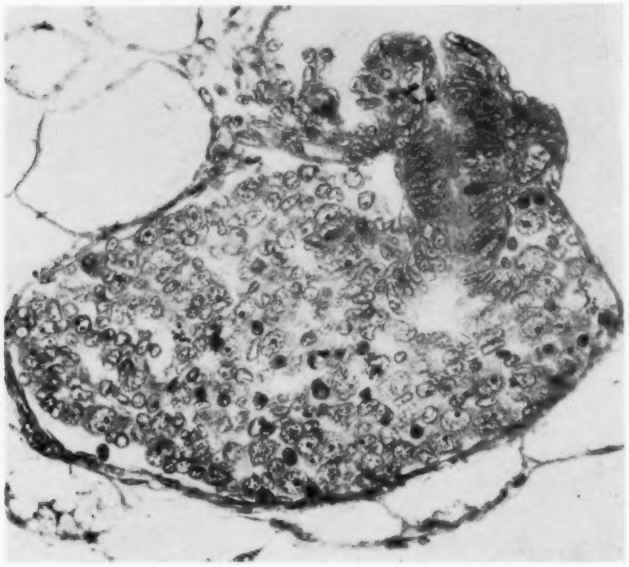


Fig. 47. Pronephros of a 10 cm specimen (10.2). With many blood elements in the central mass; short tubules.

the central mass is well developed, its cell bodies being very numerous, distributed in all parts of the mass. The most conspicuous cell elements in the central mass are large mononuclear cells (haemocytoblasts). They are found in all parts of the central mass but most abundantly around the central cavities. They are surrounded by fine fibres belonging to the fibrous tissue. These fibres form dense capsules around the mononuclears. Some of the mononuclears divide, and as a result of these cell divisions some medium-sized mononuclears are found in the fibrous substratum. A few overfed large mononuclears form giant cells. A tripolar division spindle in such a cell indicates that these cells are megacaryocytes. No cells—excepting lymphocytes—smaller than the medium-sized mononuclears are found in the fibrous network, nor was a medium-sized cell found in division. The location in some sections of numerous haemocytoblasts around the bases of the tubules indicates that they develop there. In reality it is easy to convince oneself that "formative cells" of the tubules emigrate from them to join the central mass as haemocytoblasts. By this process the cells are surrounded by transformed columnar cells, which may form a sort of capsule around the "formative cell." In other cases the mononuclears arise between columnar cells already transformed into young fibre cells and are carried by these into the central mass. At the surface of the central mass cell bodies belonging to fibre cells form a sort of incomplete

Fig. 48. Pronephros of a 10 cm specimen (10.3). Blood formation in progress; short tubules.

covering of the mass. It has been frequently stated that such cell bodies become detached from the mass and move freely into the pronephric vein, forming lymphocytes. Some of these cells have retained a small part of their fibre and form typical spindle cells, numerous in the blood of *Myxine*.

"Formative cells" of the tubules thus develop into blood elements. But not all the formative cells go through this development. Others retain their place in the tubules, divide twice and then become common columnar cells.

Head kidney 10.2 and 3. This kidney differs from the preceding in its tubules being relatively short, mostly containing but a few formative cells. The central mass is very bulky, causing a saclike swelling of the pronephric vein. The shortening of the tubules is apparently due to the haemopoietic activity of this kidney, their basal part having been consumed as its contents of formative cells have been poured out into the central mass, causing its increasing volume. In some tubules, however, the production of large mononuclears is still going

VOL. 31 1950

on, but for the most part this activity has ceased. The central cavities are generally small, but show a tendency to increase their size, joining adjacent vacuities in the central mass. The central mass has not the dense appearance of the preceding kidney, as its denser parts are located on the surface that borders on the pronephric vein. The central part of the mass is composed of looser matter, with vacuities of different sizes in which the cell elements are dispersed (Fig. 47). The fibrous tissue apparently dissolves in the central part, its cell bodies there being detached from their fibres. At the surface of the mass fibres are seen between the densely aranged cells, but it was impossible to discover whether or not these fibres are merely remains of the original set of fibres. As there are very clear signs that cell bodies belonging to the fibrous tissue are set free at the surface of the mass to join the blood stream as small lymphocytes or spindle cells, I must presume that many of the fibres are degenerating; for degeneration must necessarily take place when the blood elements have passed into the pronephric vein. The formation of new fibre cells is only seen in a few places at the base of tubuli. In the degenerating meshes of the fibrous tissue lie the blood elements (Fig. 48). They are large (haemocytoblasts), medium-sized and small, rounded mononuclear cells, megacaryocytes, lymphocytes and phagocytes. At the surface of the central mass different-sized mononuclears are seen detaching themselves from the mass and moving frely into the lumen of the pronephric vein. The phagocytes—probably derived from the fibrous tissue (or columnar cells)—are found in the central cavity and in the larger vacuities. These cells sometimes have a comparatively large amount of cytoplasma and a more or less irregularly formed nucleus.

In the interior of the central mass, especially where it is rather dense, a few red blood corpuscles are visible.

The activity of this kidney may still not be very important and the amount of blood elements delivered to the circulating blood can not be very large. The process here must have been a rather slow one and the blood elements may be poured out quite gradually so long as immature blood elements are still present in the central mass.

Head kidney 10.4 (Fig. 49), 10.5 (Fig. 11) and 10.6 (Fig. 50). These three kidneys apparently belong together. The production of haemocytoblasts is going on in some of the tubules at least, and great masses of large mononuclears are delivered to the central mass. The fibrous tissue is weakly developed and the blood elements form a loosely buit-up tissue, where the blood cells are not held together by any regular network of fibres. The blood elements are mononuclears of three sizes, some leucocytes and many lymphocytes. The scarcity of fibre cells and the abundance of large blood elements give the central mass a large-celled appearance. In the rather broad central cavities phagocytes are present. Red blood corpuscles are common in the central as well as in the peripheral part of the central mass. At the anterior end of the central mass a lively

Fig. 49. Pronephros of a 10 cm specimen (10.4). Central mass pouring its contents into the pronephric vein. Fibres in parallel rows through the central mass.

VOL. 31 1950

emigration of blood elements is in progress, and there all classes of cells are delivered in large quantities to the pronephric vein (Fig. 33). Longitudinal fibres form long parallel strands, between which the blood cells seem to move out into the vein. These strands are ultimately broken up into short portions, each containing a cell body. Such cell bodies with fibre fragments form spindle cells. Other cell bodies of fibre cells form lymphocytes, which may contain one or two small nuclei or an irregularly formed nucleus.

The occurrence of red blood corpuscles in the central mass already seems to indicate that erythropoiesis occurs in the mass, as has already been described in a separate chapter.

The amount of blood elements produced by these kidneys seems to be very considerable, especially as the haemopoietic process seems to be sustained by the formation of new haemocytoblasts from at least some af the tubules. These are located—in these three kidneys—in the posterior part whereas the delivery takes place at the anterior end of the kidney. On account of this location the delivery period is likely to be a prolonged one.

Head kidney 10.7. This kidney is peculiar as its anterior half has tubules with but very few formative cells. The anterior half of the central mass is retained at a stage of development corresponding to that of the head kidney 10.5, as described before. The posterior half, on the contrary, has tubules with numerous formative cells, and its central mass portion is in a vivid proliferation period with blood elements of every description, which, however, are not yet

Fig. 50. Pronephros of a 10 cm specimen (10.6). Central mass pouring its contents into the pronephric vein.

emigrating into the pronephric vein. Possibly the anterior part of this kidney may already have delivered its contents into the vein and the posterior has not yet begun this process. In the anterior part the blood elements there present are no doubt the remains of a preceding haemopoietic activity; but they may also be explained as signifying an individual variation.

Head kidney 10.8. I consider this kidney to be at the end of its haemopoietic activity or at the beginning of a regeneration period, which is the same thing. The tubules are long, with very few large cells between the columnar epithelial cells. The central cavities are large, joining great vacuities of the central mass. The central cavities almost invariably contain phagocytes forming clusters or moving freely in the cavities. The central mass has a spongious structure, with open spaces of varying size, in which numerous phagocytes and lymphocytes are collected. Such spaces were occasionally found opening into the pronephric vein and pouring out their contents of phagocytes and lymphocytes into the vein. At the surface of the mass a not insignificant number of haemocytoblasts and medium-sized and small mononuclears as well as lymphocytes are distributed together with cell bodies belonging to the degenerating fibrous tissue. All these superficial cells seem to belong to a haemopoietic period already passed. In some sections megacaryocytes and haemocytoblasts were degenerating, with nuclei in which the chromatin has changed from basichromatin to oxychromatin. Probably such cells were about to be dissolved. A very striking occurrence in this kidney is that a new set of fibre cells is

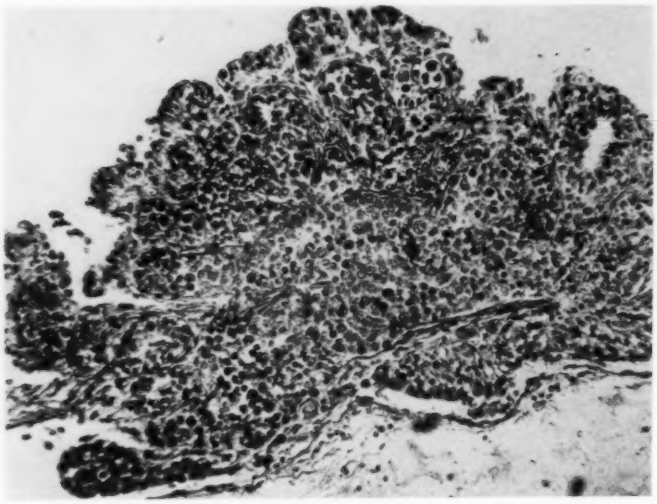


Fig. 51. Pronephros of a 10 cm specimen (10.9). Excessive formation of blood elements. Tubules mostly closed.

developing at the bases of the tubules and in the spaces between adjacent tubules. There is thus a regeneration of the fibrous tissue, replacing the older degenerating set. As soon as this degeneration is brought to an end, the new set will dominate the central mass.

Head kidney 10.9. The pronephroi of 10—12 cm specimens hitherto described form a tolerably well-established series of the haemopoietic activity. However, the pronephros 10.9 cannot be included in this series, but must represent a side line of the "normal" pronephric activity. I believed at first that this kidney was quite anormal, but as I have met with practically the same type in three other specimens of different sizes, I must regard it as a rare but nevertheless a normal occurrence. The 10.9 kidney may be described as follows.

The kidney is very thick. The tubules do not protrude into the pericardial cavity. Only their mouthpieces are seen on the surface of the kidney. They are very large, forming thick discs (Fig. 51). However, where the stomata should be, the tubules are generally closed. Only a small number of the tubuli have a typical columnar epithelium. In most of the tubules the columnar cells have been transformed into masses of blood elements of some kind. These cell masses have caused the obliteration of at least some parts of the lumen of the tubules. In some cases the canal has completely disappeared. As these cell masses are apparently poured out into the central cavities without any distinct boundary, one may say that the tubuli form a part of the central mass. The central cavity is thus completely filled up with these cells, and consequently its

VOL. 31 1950

primary walls have been greatly distended by the great quantity of blood elements poured out into it. The cells which fill up the tubuli and the central cavity are the usual blood elements: mononuclears of all sizes and phagocytes. In addition, there are also degenerating cells with pycnotic nuclei in the centre of the tubule. Fibre cells are only present in connection with trabeculae piercing the central mass and in the thin layer forming the walls of the central cavities. In consequence the blood elements are not enclosed in a typical network formed of fibre cells. The central mass proper thus consists of the rather compact cell layer (formed by fibre cells) around the blood cell mass. In the centre of this mass degeneration apparently takes place, causing the formation of a vacuity in which blood elements of different kinds are floating. Many of these cells are degenerating, as are also a great many cells in the surrounding compact part of the central mass. Phagocytes are very common, forming (in parts already degenerated) fields in which they lie pressed closely together. The cells thus have polygonal outlines. The cell body is large, containing great vacuoles; the nucleus is small, sometimes pycnotic. Red blood corpuscles are sometimes met with in the vacuities. They must have originated there, but in what way could not be disclosed.

In the posterior part of this kidney the tubules have still columnar cells, but there is apparently a degeneration in progress, as large portions of the cells have oxychromatic nuclei and are misformed in many ways. In such tubules only the basal part seems to serve as a haemopoietic centre.

In this kidney, the formation of blood elements and the degeneration of such elements and tubuli are in progress, while at the same time large quantities of blood elements are being poured into the blood stream. But with them great quantities of waste matter from the degenerative process also leave the central mass. The degeneration of the entire tubules must be followed by a regeneration. This may take place from the mouthpieces, which do not degenerate and which always contain columnar cells. Cell divisions present in the mouthpieces may be regarded as signifying such a regeneration.

Head kidneys of 11 cm specimens.

Out of 27 pronephroi of this stage at my disposal, eleven (40 %) were in haemopoietic activity in stages corresponding closely to the initial stages of the 10 cm animals already described. Two stages are near the peak of such activity, with shortening tubuli and an increasing number of formative cells in them. The other specimens belong to a fibrous type. Kidney 11.1 (Fig. 52) is built up in the same way as kidney 10.1, with long tubuli, but without formative cells, with a spongious central part and with only a very few large blood elements on its surface. New fibre cells are being formed at the base of the tubuli. Kidney 11.2 is in very close agreement with the 11.1 (Fig. 53). In the pronephros 11.3 the tubuli are unchanged, but the central mass has

Fig. 52. Pronephros of an 11 cm specimen (11.1); long tubules.

apparently shed its central and cortical parts and is now composed only of an augmenting set of new fibre cells. The volume of the central mass has accordingly decreased considerably. This kidney now seems to be ready to receive an invasion of blood-forming cells.

Sixty percent of the kidneys of this class are purely fibrous.

Summary and conclusions regarding the 9.6—11 cm stages.

In the preceding description the head kidneys have been arranged so that they form a series which obviously is an evolutional or functional series. The 9.6 cm stage—the youngest of the series—does not, however, signify a true initial stage of the functional process, as the formation of blood elements has already begun. But as these elements are comparatively scarce in the central mass and the long tubules show but a comparatively small amount of formative cells, this kidney may represent an early stage of the process. It could hardly be an end stage of a preceding process, as large mononuclears are scattered over the entire mass and are also found at the base of such tubules as contain formative cells.

In the 10 cm stages described previously the amount of formative cells in the tubuli is gradually increasing and a large amount of blood elements are consequently formed, filling up the central mass. This process is correlated with a gradual shortening of the tubuli, due to their basal parts having been absorbed into the central mass. By this shortening process branches of the tubuli are brought into contact with the central mass, so that more than one tubulus may open into a single central cavity. Now the central mass fills up

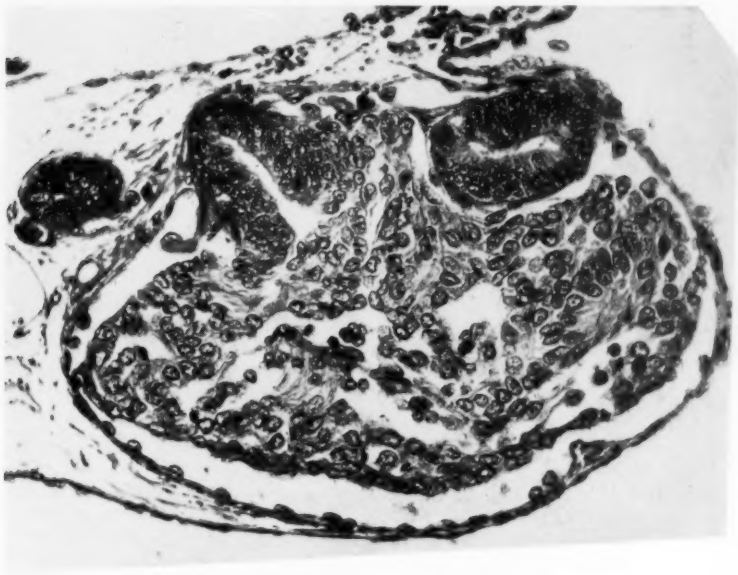


Fig. 53. Pronephros of an 11 cm specimen (11.2). Beginning of a new active period; regenerated fibrous tissue.

the pronephric vein completely. The formation of blood elements is very vivid. Large and medium-sized and small mononuclears and also leucocytes and lymphocytes are plentifully represented in the mass, and the formation of red blood corpuscles is apparently going on within it.

In the kidneys 10.2 and 10.3 the blood elements are delivered to the pronephric vein to join its blood stream and in the kidney 10.8 this process is brought to an end, only a few blood elements being left in the central mass. Formative cells have become scarcer in the tubuli, which are increasing in length. The fibrous tissue is degenerated, but new fibrous tissue i forming. At length—in one 11 cm stage—the kidney is empty of blood elements and a regeneration of the fibrous tissue is going on. In another 11 cm stage the kidney has shed its earlier set of fibrous tissue and has developed a new set from the basal parts of the tubuli.

The delivery of the blood elements to the pronephric vein is sometimes preceded by a degeneration of the fibrous tissue surrounding them. Between the blood cells there are seen capsule cells with pycnotic nuclei and basophilous cytoplasma, indicating a degeneration of these cells. In some cases the delivery seems to be somewhat violent, as the fibres are extended into the direction of the stream of the blood cells leaving the central mass. In such a case most of the blood cells lie free in the mass, the capsules being dissolved. In these cases,

however, a regeneration of fibre elements is already beginning at the bases of the tubules.

During the process summarized above phagocytic cells are present. They are formed of fibre cells cut off from their fibres or by columnar cells emigrating into the central cavities. Those of the central cavity have to catch particles carried by the ciliary movements of the columnar cells from the pericardial cavity to the central cavity. Remains of fibrous cells may also be phagocyted by similar cells.

In the 9.6—11 cm stages one function of the pronephros is thus largely to form blood corpuscles—white and red. Another function must be to filter the fluid of the body cavity and to conduct the filtered fluid into the circulating system. As the pronephros has no outlet to the exterior, its excretory function cannot be very important. Some of the excretory products of the pronephric glomus (glomi), which are delivered into the body cavity, may enter the tubules and then through the central mass come into the pronephric vein and thence to the heart. Such products may then be conveyed to the mesonephros and from there to the exterior. Another part of the excretory products, collected into the body cavity, may be emptied through the abdominal pore.

As it is not probable that the blood-forming function in all specimens of 9—11 cm body length begins and ends at the same time, it may be expected not always to have ended in specimens with a body length of 11 cm. In my collection I have two specimens of 11 cm body length, in which the central mass is nearing the height of its blood-forming function. Such a displacement of the functional stages becomes more accentuated in the older stages so much so, in fact that it is no longer possible to point out without close examination which pronephroi belong to the one and which to the other period of function. In older stages, moreover, the individual variation increases very much, as has been explained above. In the 9—10 cm stages this individual variation is very small, so far as could be ascertained. Therefore the process must be much more regular than in older stages. The fact that nearly all my 10 cm specimens are involved into the blood-forming function seems to indicate that the function in these specimens is of longer duration and more intense than in the other ones.

The pronephros of 12 cm specimens.

I have at my disposal 68 head kidneys of animals of this size. In this material there are 25 kidneys (37 %) with haemopoietic activity.

Generally this activity is characterized by stages closely corresponding to those described in the 10—11 cm stages, with the formation of large haemocytoblasts in the tubules or at their base. Six of these 25 kidneys were at the height—or nearly so—of their lymphatic activity, the other 13 were either beginning or ending this activity.

VOL
31
195

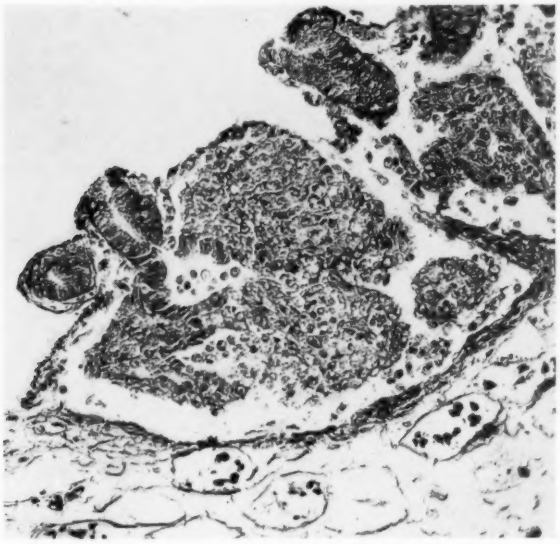


Fig. 54. Pronephros of a 12 cm specimen (12.7). A central cavity opening in the pronephric vein.

The initial stages of activity are characterized by a vivid regeneration of the fibrous tissue at the bases of the tubuli, as demonstrated in 12.9 (Fig. 9) and 12.4. Through this regeneration the central mass becomes more or less compact. Among the fibrous cells at the bases of the tubuli are also found small rounded cells (lymphocytes), which become enclosed in the fibrous network. This process may in some cases be prolonged for a certain period so that the central mass becomes a rather thick and compact cell mass, consisting only of fibre cells and lymphocytes. In the central cavities free cells are accumulated. Some of these probably act as phagocytes, causing the widening of the central cavities. Phagocytes may also develop outside the central cavities, causing the formation of other cavities in the mass, in which masses of free cells may be found. Such cavities sometimes open into the pronephric vein and pour out their contents into the blood. Fig. 54 and 2. In other cases there may also be formed between the fibre cells bigger rounded cells. These bigger cells are apparently haemocytoblasts. In kidney 12.3 they are fairly common, the lymphocytes and fibre cells, however, predominating in the main part of the central mass.

Likewise in the 12 cm stage haemocytoblasts may develop in abundance at the bases of the tubules from cells belonging to the infundibulum and the inner wall of the central cavity. In such a case the fibre cells are in the minority and the central mass becomes filled up with more or less free blood elements of

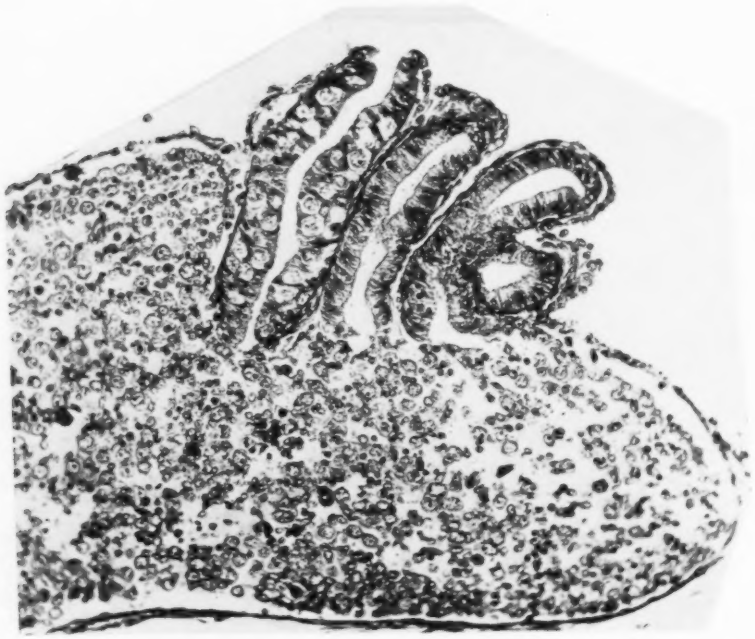


Fig. 55. Pronephros of a 12 cm specimen (12.2). Many "formative" cells in one of the tubules. Central mass loosening.

different sizes. In most cases belonging to the 12 cm stage the tubules contain no formative cells though the infundibulum is then very insignificant and the formative cells apparently develop into columnar cells. In that case they probably signify a regeneration of the tubular epithelium.

The blood cells become free through the dissolving of the fibrous capsules that may possibly surround them, as is clearly seen in kidney 12.2 (Fig. 55). After the delivery of the blood elements to the pronephric vein, the central mass becomes a slender band of more or less conspicuous fibre tissue with great vacuities containing phagocytes and lymphocytes. The regeneration of fibres then sets in and the kidney prepares for a new lymphatic activity. The period of lymphatic inactivity may be a rather prolonged one, as in about 63 % of the kidneys of the 12 cm stage there is no visible haemopoietic activity.

One single kidney among the 12 cm specimens (12.12) differs from the description of active kidneys given in the preceding lines. In this kidney many of the tubules are strongly swollen on account of the vivid cell divisions which have taken place in their columnar epithelium. The cells resulting from these divisions have a degenerated appearance, with swollen, weakly coloured cell bodies and small chromatic nuclei. Such cells are produced in great masses,

which—basally in the tubes—are poured out into the central cavity of the central mass. In some cases the envelop of such cell masses has broken and the contents are about to be conveyed to the pronephric vein. In such cases the cells become freed from one another and then appear as elipsoid bodies, with vacuolated cytoplasm and a rather small nucleus. In that stage they cannot be distinguished from phagocytes.

Besides these cells, however, there are normal blood elements present, viz. a few haemocytoblasts, many medium-sized mononuclears and lymphocytes. Degenerating cells belonging to these types are also common. In this kidney the swollen tubules always have a short apical part of normal appearance and with stomata opening into the pericardial cavity.

This kidney very closely resembles kidney 10.9 (Fig. 51) of the 10 cm specimen, described previously, but the products of the cell divisions in the tubuli are different, as they appear from the very outset to have been degenerating.

OL.
31
950

Head kidneys of 13 cm specimens.

In 60 head kidneys belonging to this class eleven (18 %) were found to be in stages of haemopoietic activity. The remaining 49 were either recovering from an earlier blood-forming activity or preparing for a following period of such activity.

Head kidney 13.2 (Fig. 34) is at the beginning of its activity. In some of its tubuli big formative cells are present. But most of such cells are located at their infundibula, where a vivid proliferation of big haemocytoblasts takes place between the fibre cells. The central mass forms a slender band floating into the pronephric vein. It consists of typical fibre cells surrounding rounded spaces, in each of which generally one blood cell is enclosed. Besides the haemocytoblasts, medium-sized mononuclears (proerythroblasts) are present. These are formed by division of the haemocytoblasts. However, a few leucocytes were found. Megacaryocytes are present in great numbers. Pluripolar mitoses are not rare in the megacaryocytes.

In kidney 13.6 the anterior part has a central mass of the same type, but its posterior part is quite fibrous.

Kidney 13.7 (Fig. 56) is very interesting as it is closely reminiscent of kidney 10.9 and of the last-mentioned of 12 cm. The central mass of kidney 13.7 is rather large, consisting chiefly of large cells (haemocytoblasts, megacaryocytes and medium-sized mononuclears) surrounded by fibres belonging to fibre cells, the small cell bodies of which are distributed among the larger cells. The central mass contains large vacuities partly filled with free elements or islands of such cells. These are of different sizes (haemocytoblasts, medium-sized and small mononuclears, lymphocytes, leucocytes and phagocytes). Numerous cells with degenerating pycnotic nuclei are seen in the vacuities.

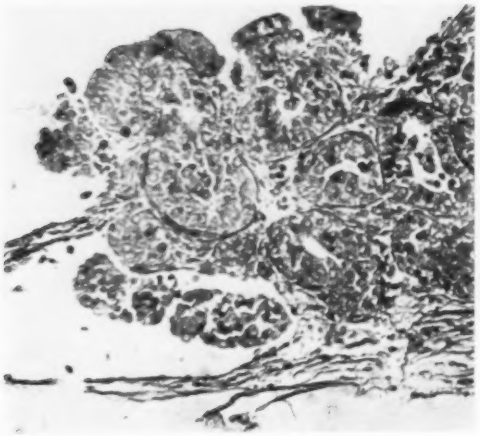


Fig. 56. Pronephros of a 13 cm specimen. Tubules obliterating their canals.

VOL.
31
195

Further, some red blood corpuscles may occur not only in the latter, but also in the central mass proper. In some places the central mass delivers blood elements to the pronephric vein. These cells are mostly medium-sized mononuclears (proerythroblasts), spindle cells and lymphocytes.

The most striking feature of this kidney is, however, the behaviour of the tubules, in which typical columnar cells are found only in the closed mouthpieces, the columnar cells of the tubules proper and the infundibular wall having, figuratively speaking exploded into the formation of blood elements, mostly haemocytoblasts and medium-sized mononuclears. However, deformed columnar cells also occur between the blood cells. The differentiation of the blood cells has apparently been sudden, so that the lumen of the tubules has disappeared completely, especially in their external parts. In the basal part, however, a lumen is present but it is filled with large more or less free rounded or pear-shaped cells pouring out into the wide infundibular parts of the central cavities. These cavities are continuous with the large vacuities of the central mass. Thus a mighty stream of young blood elements pours out from the tubules into the central mass, causing its increasing size. In both kidneys of this specimen there is not a single tubule of normal aspect. The multiplication of the cells of the tubules and the central mass is mitotical. I have found no certain traces of amitotical cell division.

In kidney 13.3 (Fig. 57) a small number of tubules behave as in 13.7, whereas the rest of them are typical with normal columnar epithelium. The central mass is strongly developed, frontally delivering masses of typical blood elements to the pronephric vein. This kidney may possibly have once been of the same type as kidney 13.7 but is now partly recovering from a period of

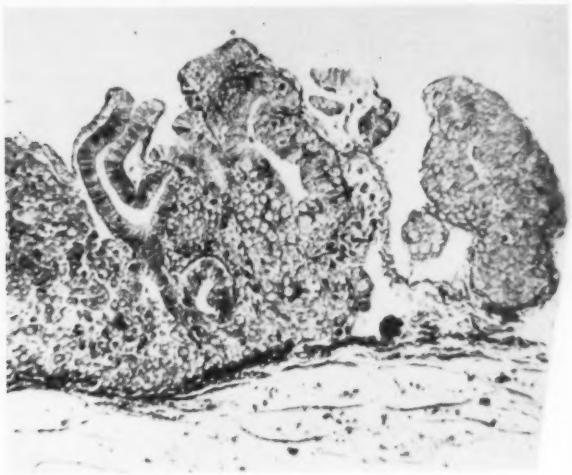


Fig. 57. Pronephros of a 13 cm specimen (13.3). Tubules thickened through formation of blood elements.

OL.
31
950

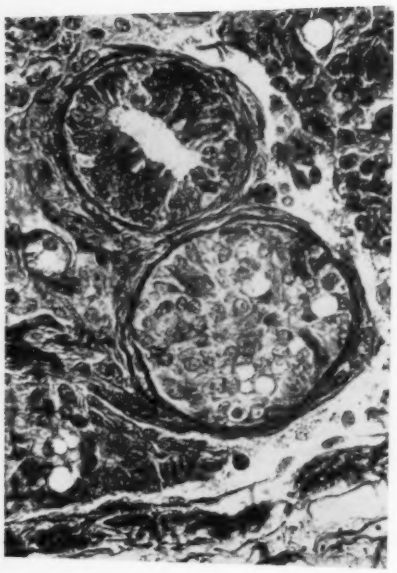


Fig. 58. Pronephros of a 13 cm specimen (13.4). Tubule without lumen, phagocytes forming in the wall of the tubule.

Fig. 59. Pronephros of a 13 cm specimen (13.5). Central cavity with two tubules; fibrous type.

excessive haemopoietic activity. The same may be true of kidney 13.4 (Fig. 58). In this kidney some of the tubules are solid, consisting of a mass of mostly small cells, part of which are conveyed as small cells (phagocytes) to the central mass and another part is used up for the regeneration of the columnar epithelium of the tubules. The central mass is but little developed, without large blood elements. This kidney is probably at the end of a period of haemopoietic activity.

As already pointed out, most of the 13 cm kidneys are fibrous, recovering from a preceding or preparing for a future period of activity. The development of fibre cells is the most striking feature of these kidneys. These cells develop partly at the bases of the tubules, partly from cells covering the surface of trabecles. In 13.6 such developing fibre cells are seen collected in bundles on a coarse horizontal trabecle. In 13.5 is illustrated a case which becomes very common in larger animals, where two or more tubules may open with a common infundibulum in a single big central cavity (Fig. 59). This occurrence is the consequence of a reduction of the basal part of a biramous tube, with the result that the common part of the two tubes has disappeared and the two tubes have thus each acquired an opening into the central cavity.

VOL.
31
195

Head kidneys of 14 cm specimens.

In 43 head kidneys of this class there are seven[1] (15.5 %) showing at least some haemopoietic activity. In six of the cases, however, this activity is very inconsiderable, with only a few large cells in each kidney. Though in some of them formative cells are present in the tubuli (in one instance in considerable numbers). These cells divide at least twice without leaving the columnar epithelium, and the cells resulting from these divisions are apparently transformed into columnar cells. These formative cells thus signify a reconstruction of tubuli after a period of haemopoietic activity. This conclusion is in accord with the appearance of the central mass, where a vivid development of the fibrous tissue is going on at the base of the tubules and along the trabecles, simultaneously with a central destruction of tissue elements remaining from the preceding period of activity. A few haemocytoblasts and other blood cells are still present, remaining from the preceding period. The destruction issues from the central cavities, which are enlarged by the addition of vacuities in the central mass, left when the blood elements were emitted from it. These cavities contain many phagocytes, lymphocytes and also, sometimes, red blood corpuscles and waste products.

In one kidney (14.2) only, belonging to a specimen of 14.5 cm body length, there was a vivid production of blood elements, mostly medium-sized and small mononuclears (but also haemocytoblasts and phagocytes). These elements

[1] If those kidneys in which the activity is very small are not counted, the percentage will be about 2.

Fig. 60. Pronephros of a 15 cm specimen (15.6).

were produced in the tubuli, in at least the basal ⅔ of which the columnar epithelium was substituted by rounded cells filling up the wall of the tubule so as to cause its lumen partly to disappear or become comparatively narrow. The rounded cells of the tubules are poured into the central mass. In this mass destructive processes are at work, issuing from the central cavities. This kidney thus very closely recalls kidney 13.7 and others described in preceding pages.

Those kidneys in which the central mass is quite fibrous are so variable in appearance that no description applicable to them all can be made. In some of them the central mass is very poorly developed, forming a thin band of tissue at the base of the tubules; in others the formation of new fibrous tissue has made the central mass much more voluminous. However, it always contains more or less pronounced vacuities, mostly connected with the central cavities and with the same contents of phagocytes and lymphocytes and waste products as the latter.

Head kidneys of 15 cm specimens.

In 47 specimens of 15 cm body length there are but six (13 %) containing head kidneys with blood cells of the different classes. Two or three of these kidneys apparently belong to a preceding period of activity as their central mass contains large vacuities and but few large cells collected at the surface of the mass. Simultaneously, however, there is a slight haemopoietic process

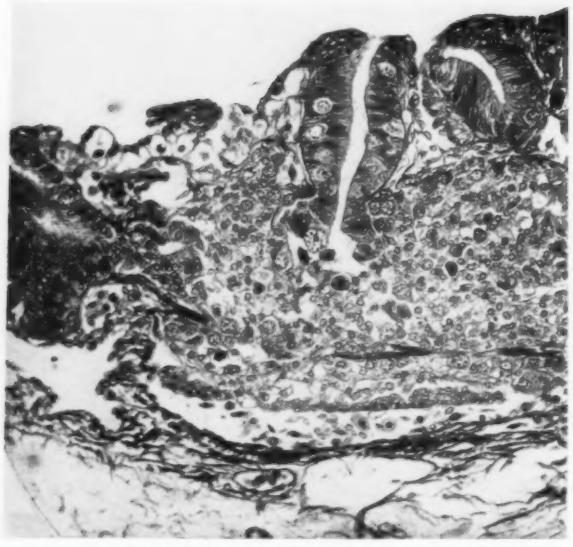


Fig. 61. Pronephros of a 15 cm specimen (15.5).

VOL.
31
1950

going on at the base of a few tubules, in which formative cells are present. New fibre cells are also forming at the base of the tubules.

In another specimen (15.6) (Fig. 60) the activity is somewhat advanced and the still slender central mass contains a great number of haemocytoblasts distributed all over it. The vacuities, still distinct, are becoming filled with fibre cells and haemocytoblasts developing at the base of the tubuli. Clusters of phagocytes are present in the central cavities.

Specimen 15.5 (Fig. 61) is nearing the height of haemopoietic activity. In many of its short and thick tubules a lively production of formative cells is in progress, and the central mass contains numerous large blood elements. In addition, the forming of haemocytoblasts and fibre cells from the infundibula is in progress. The fibre cells are difficult to recognize in that part of the central mass lying inside the rather small central cavieties, as their fibres are swollen and disintegrating, preparatory to the formation of vacuities in the central mass. This condition is not an artificial one produced by the technical treatment of the kidney, as the fibres are clearly visible in the posterior part of the same kidney, where the development is not so far advanced as in the rest of it. A large number of haemocytoblasts develop from cells on the surface of trabecles piercing the central mass.

All other kidneys belonging to the 15 cm specimens are of a pure fibrous type either preparing for or recovering from a preceding period of activity.

Head kidneys of 16—16.5 cm specimens.

In 76 specimens of 16 and 16.5 cm body length there are twenty-one (28 %) present which have large blood elements in their head kidneys. Fifteen out of these 21 kidneys have but a few large cells in the central mass. They are located either near the infundibulum or on the outskirts of the central mass. In the former case they may be newly formed, in the second they have probably survived from an earlier proliferation. In one of these kidneys formative cells were present in some tubuli.

One of these 21 kidneys belongs to an animal of 16.5 cm body length. This kidney exhibits some interesting features worthy of mention, as they have not been met with hitherto. The kidney seems to have passed beyond the main part of its functional development and the degeneration has set in and gone on so far that great vacuities are connected with the original central cavities. These vacuities are filled with free or loosely connected cells of different sizes and qualities. Some of them are apparently fibre cells or detached cell-bodies of fibre cells. Others appear to be cells that have become freed from their fibre capsules. The smaller of them represent mononuclear and binuclear lymphocytes, the larger are mononuclears of larger sizes. Many of the smaller cells seem to have increased their plasmatic parts, enclosing vacuoles and granules; these are phagocytes. The central vacuities are surrounded by a rather dense cell mass, in which a partly dissolving fibrous network, containing rounded cells, is discernible. The rounded cells are mononuclears of the three different sizes. Some megacaryocytes are also present. These cells contain fat drops of different sizes. The fibre cells may likewise contain such drops (Fixation in Flemming's fluid). Similar drops are also found in free spaces between the cells. This area of denser tissue extends to the free surface of the central mass, bordering it towards the vein lumen. There, however, the density increases. This surface portion of the central mass is in process of dissolving. The capsule cells are disintegrating, their contents being shed into the vein lumen. The large cells contained in the capsules are also degenerating and their fat contents are set free into the vein lumen, which thus contains masses of fat corpuscles. Between fibre cells running parallel with the surface are seen fat corpuscles, and fat is also present in the fibre cells. There thus seems to be a general fatty degeneration taking place at least in the surface parts of this kidney. As haemocytoblasts are present at the infundibulum, a regeneration of blood elements may occur simultaneously.

Head kidneys of 17 cm specimens.

In 32 specimens of this class there are 12 (37 %) exhibiting at least traces of haemopoietic activity. Of these, three are at the very beginning of this activity, having the basal part of at least some of their tubuli swollen with

formative cells or blood elements, of which, however, only a small number have entered the central mass, which is quite slender and fibrous throughout. Four of the other kidneys have apparently reached the end of a period. Their tubuli contain no formative cells. The central mass is fibrous, with many vacuities and a few scattered haemocytoblasts and smaller mononuclears, especially at its surface. One single kidney seems to be at the height of its activity with a large bulky and compact central mass filled with blood cells of every size. This kidney, however, cannot be analysed more closely, as it is too badly preserved to allow of a detailed description.

All other kidneys are quite fibrous without a single large cell. The central mass contains many large vacuities more or less filled with phagocytes and lymphocytes. These cells are conveyed to the pronephric vein when the surface layer of the central mass is broken through, as is the case in 17.1.

Head kidneys of 18 cm specimens.

In 29 specimens of 18 cm body length eight of the kidneys (28 %) contain haemocytoblasts. In seven of them only a few such cells are present. These kidneys were apparently at the very end of a haemopoietic period. The eighth, however, was at the highest point of haemopoiesis. The central body is filled with blood elements of every description. The central parts of the body are already loosened, preparatory to the conveyance of the cells to the pronephric vein. This kidney (Fig. 27) contains all the kinds of blood elements present in the circulating blood and was especially used for the description of the development of the different blood cells. See page 274. All other kidneys were fibrous throughout with large vacuities in the bandlike central mass.

VOL.
31
1950

Head kidneys of 19 cm specimens.

In the kidneys of these animals eleven (36 %) out of 30 demonstrate at least some haemopoietic activity. Of these seven kidneys, one is at the beginning of a period, two near its climax, one at the climax and the remainder at the very end of a period of activity. All other kidneys are fibrous throughout. Kidney 19.3 is at the beginning of a period. Some of its tubules contain a large number of formative cells, of which the basal ones are streaming out as haemocytoblasts into the central mass, where they are already very numerous. Generally, however, they have not begun dividing, so that these large cells predominate in the central mass. Large vacuities connect up with the central cavities and also with one another. In the vacuities lymphocytes, leucocytes and phagocytes are present in considerable numbers. Some haemocytoblasts were also found there. Red blood corpuscles were found in the vacuities. In such cases the vacuities were in communication with the pronephric vein.

In the two kidneys with high activity (19.1 and 19.2 [Figs. 62, 63]) the tubules consist of columnar cells only, and the central cavities are confluent

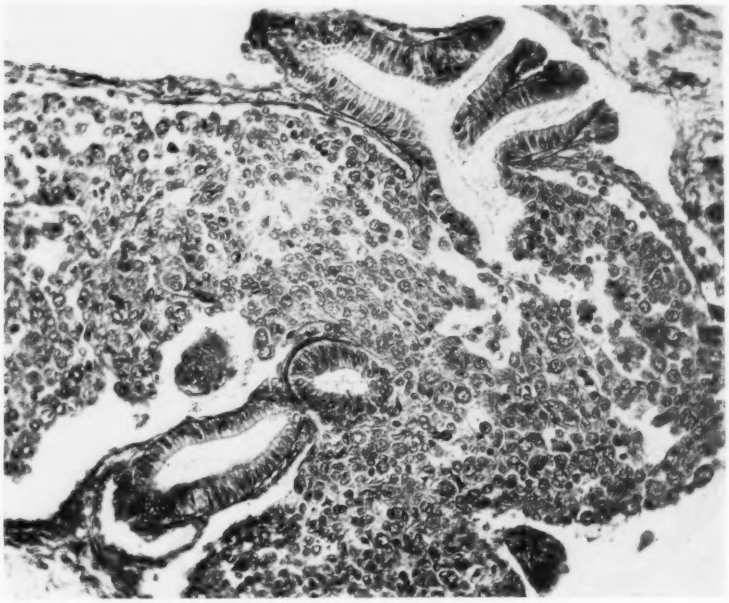


Fig. 62. Pronephros of a 19 cm specimen (19.1).

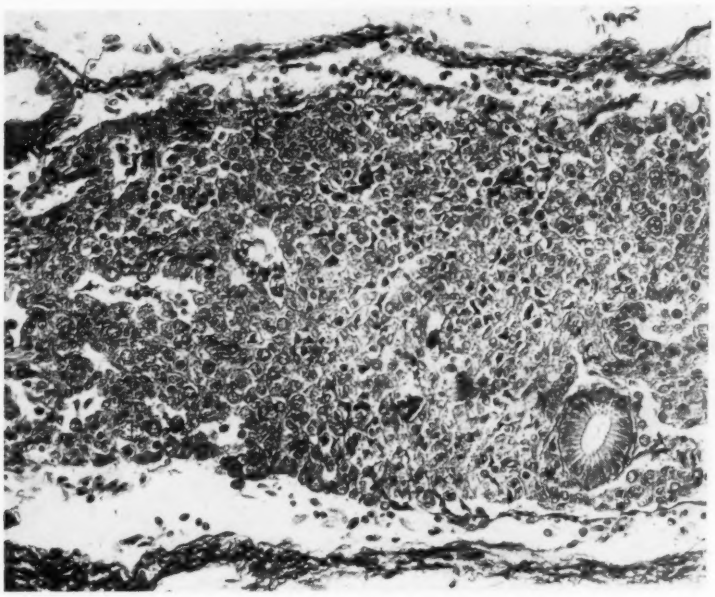


Fig. 63. Pronephros of a 19 cm specimen (19.2). Capsule cells degenerating (black in the picture). Such cells are delivered in chains to the pronephric vein (below to the right).

with large vacuities inside them. These vacuities have apparently arisen through the partial destruction of the fibrous cells present earlier in the corresponding spaces. There remains, however, a wide plexus of fibre cells containing in its meshes numerous free cells, lymphocytes and phagocytes, as well as remains of fibre cells. Cells of larger sizes are also present in these loosened central parts of the central mass. Around this spongious central part the tissue is denser, containing very numerous blood elements of every description. In parts of kidney 19.1 (Fig. 62) these elements are surrounded by fibre capsules with well-defined fibres. Especially in the superficial portions of the central mass the fibres are swollen and fused on to one another, forming structureless capsules around the blood cells. Such capsules seem to dissolve at the very surface of the mass, shedding their contents into the pronephric vein. In specimen 19.2 (Fig. 63) the picture is partly different. There the entire central mass is loosening. The fibrous network is largely degenerating, partly swollen, partly transformed into basophilus cellular remains, where a pycnotic cell nucleus is sometimes discernible in the darkly stained cell bodies. This degeneration of the fibrous tissue sets the blood elements free. At the surface of the central mass long rows of degenerating fibres may float into the lumen of the vein, which also contains free blood elements.

In specimens 19.1 and 19.2 the central cavities are very large, with a variable number of tubules opening into them. This condition usually arises when at the height of activity the basal part of the branching tubule is consumed up to the branching point, pouring out its contents of blood elements into the central body. In this way the branch acquires an opening directly into the enlarged central cavity. Another way of widening the central cavities occurs in kidney 19.1. In that case, cells on the surface of a trabecle arrange themselves into an epithelium-like disc. Such discs may represent initial stages in the development of new infundibula, independent of tubules. The epithelial cells are provided at an early stage with cilia extending into a free space inside the disc. This free space represents a new central cavity, which either joins on to an adjacent cavity or develops a tubule of its own. Normally the trabecles (of this stage) are surrounded by short radiating fibre cells. The epithelial discs arise when cells which normally form fibre cells connected with a trabecle develop into columnar cells.

Head kidneys of 20 cm specimens.

Of the 27 specimens belonging to this group only three (11 %) exhibit some traces of haemopoietic activity. One of these three is at the very beginning of such activity, with formative cells in some of the tubules and with haemocytoblasts at their bases; the other is at the end of a period, with many large vacuities in the central mass and with but a few large elements at the surface of it—remnants from a past period. All the other kidneys are fibrous.

VOL. 31 1950

Head kidneys of 21 cm specimens.

Only four (19 %) of the 21 kidneys of this class belong to an activity period. Three of them are characterized by a great number of haemocytoblasts forming at the bases of the tubuli and in the surroundings of thick trabecles. The tubuli are devoid of formative cells. Medium-sized mononuclears, lymphocytes, leucocytes, phagocytes and megacaryocytes are also present. These three kidneys have not yet attained the height of activity. The fourth is at the very end of an active period. All the other kidneys are fibrous.

Head kidneys of 22 cm specimens.

Of 16 specimens belonging to this class two are at the beginning of their period of activity, two near the height and one is at the very end of it. There are thus 5 kidneys (31 %) with haemopoietic activity.

Head kidneys of 23 cm specimens.

Out of 22 head kidneys of 23 cm specimens ten (42 %) exhibit haemopoietic activity. Of these ten, five are in strong activity, but have not yet attained the climax, two are at the height of activity, shedding their blood cells into the pronephric vein, and two are at the very end of the period of activity. One of these ten kidneys is very peculiar. Its central mass is greatly reduced, containing only haemocytoblasts in its anterior portion, whereas its posterior part apparently has not begun its activity. The kidney has, especially in its posterior part, quite normal tubules, without formative cells. At the medium and anterior parts some of the tubules are very strongly altered, their columnar epithelium having divided repeatedly, so that there is not a single typical columnar cell left. The lumen of the tubules is partly obliterated by the great masses of rounded cells produced by the cell divisions. These cells are of every size from the great formative cells to small lymphocytes or phagocytes. At the bases of such tubules the cells have small nuclei, distributed in a plasmatic mass and containing large rounded vacuoles. This mass has about the same aspect as those parts of normal kidneys lying inside the central cavities, for instance in the 15 cm stage, and which is destined to desintegrate, causing the formation of big vacuities filled with phagocytes. Kidneys of this type have been met with in the following stages: 10.9, 12.10, 13.3, 13.4. I believe therefore, that these tubules in this 23 cm specimen do not have any appreciable influence upon the haemopoietic activity of this kidney. All the other kidneys of the 23 cm class are fibrous throughout.

Head kidneys of 24 cm specimens.

Out of 20 specimens of this class eight (40 %) have kidneys containing large blood cells. One of them is at the beginning of its activity, another near

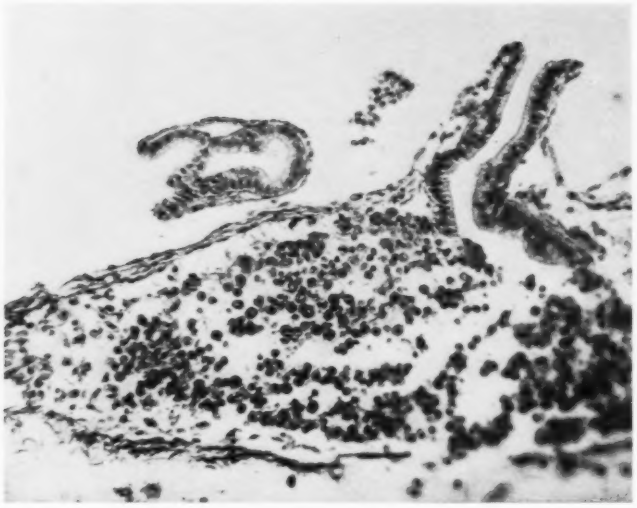


Fig. 64. Pronephros of a 25 cm specimen. Central mass loosened, cells pouring into the pronephric vein.

VOL. 31 1950

the height, and the others at the end and the beginning of the next. All the other kidneys are quite fibrous.

Head kidneys of 25 cm specimens.

Of 31 specimens only three (10 %) are in haemopoietic activity. In one of these the central mass is very bulky, filling up the pronephric vein completely. It is compact, composed of densely crowded blood cells of every kind. Another kidney (Fig. 64) of this class is just about to shed its contents in the vein and the third is shedding the last remains of blood elements.

In one of the kidneys the superficial collagenous matter of the trabecles is split up into many fine threads connected with each other transversely so that the thick central bar is surrounded by a collagenous reticulum. In this reticulum small lymphocyte-like cells are enclosed. This complex is surrounded by a thin collagenous membrane. The "lymphocytes" are thus enclosed in a thin sac through which the trabecle proper runs. Some of the enclosed cells are larger than the others. They are probably phagocytes. How this blood sac has arisen it is impossible to say for certain, but it seems at least possible that lymphocytes have invaded the trabecle from the surrounding fibrous tissue. Some of the blood sacs lying near the bases of the tubules have an opening into the blood-space surrounding the tubules.

From the 25 cm stage onwards small alveoles are frequently met with lying in the denser parts of the fibrous tissue. Such alveoles are formed of small

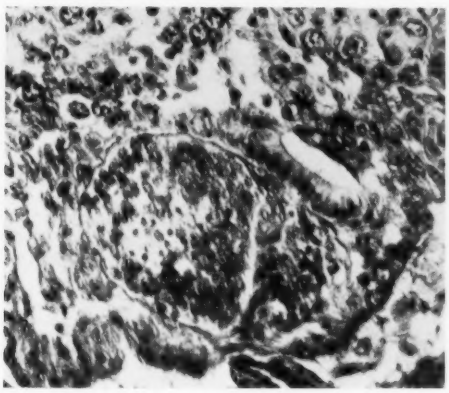


Fig. 65. Blood sac of a 27 cm specimen. Phagocytes in the centrum (white field with a few nuclei).

columnar cells arranged as an epithelium round a small round cavity. The cells may be connected basally with fibres belonging to the fibrous mass and are thus transformed fibre cells. When, as occassionally happens, the cavity is large, the cells carry cilia. These alveoles may join adjacent central cavities or serve other purposes, as has already been mentioned in this paper.

Head kidneys of 26 cm specimens.

In the 26 cm stages six kidneys (30 %) contain large blood cells, whereas the remaining 14 are quite fibrous. Of the active kidneys one is at the beginning of its activity, one has ended its activity period in its anterior part, but its posterior is highly active. Four other kidneys are at the end of their haemopoietic period.

Head kidneys of 27 cm specimens.

In this class, containing 25 specimens, seven kidneys (28 %) exhibit at least some haemopoietic activity. Of these seven, five seem to be at the beginning of an activity period, the other two at the very end of it. All the other specimens have quite fibrous kidneys.

In one of the active kidneys (Fig. 13) of the 27 cm stage a blood sac is present, which seems to represent an early stage of blood sac development. It is found round a somewhat split-up trabecle. Its lumen communicates with the pronephric vein at the angle between two tubuli. It is clothed inside with a thin endothelium—a continuation of the pronephric vein. The blood can thus apparently enter the sac directly from the vein. The sac contains only a few lymphocytes, and a red blood corpuscle is present in this sac. A similar sac in a 28 cm specimen contains lymphocytes as well as erythrocytes.

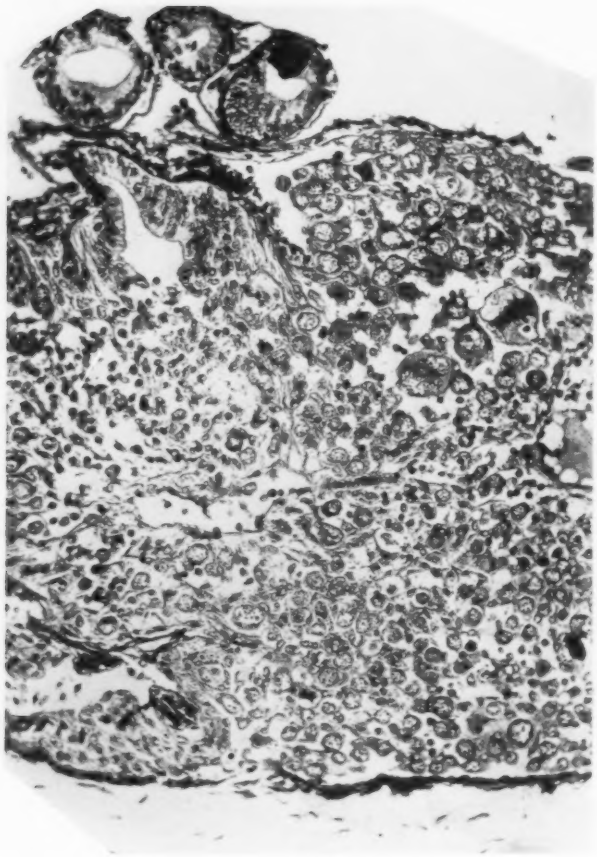


Fig. 66. Pronephros of a 28 cm specimen (28.1). Many megacaryocytes. Fibre cells developing (up to the left).

In another specimen a few big blood sacs, communicating with the venous space at the bases of the tubules contain masses of small lymphocytes round a core of bigger cells and decomposing lymphocytes (Fig. 65). The big cells are phagocytes, real macrophages, the cytoplasma of which contains fragments of the degenerating lymphocytes of their surroundings. Apparently the whole contents of the sac are going to destruction. This blood sac is analogous with a lymphatic gland, but with the difference that in the blood sac no formation of lymphocytes takes place, but only destruction through macrophages.

Head kidneys of 28 cm specimens.

Out of 31 specimens of this size, 8 (26 %) show at least some haemopoietic activity in the head kidney. In one of these (28.2) the anterior part of the

VOL.
31
1950

kidney has past its activity period, whereas the posterior is at the height of it, shedding its contents into the pronephric vein. In the specimen 28.1 (Fig. 66) the head kidney is in very strong activity, forming masses of young haemocytoblasts between the tubules and around the trabecles. The central cavities are small, but inside them there is a vast central portion of the central mass, where the tissue is loosened preparatory to the formation of large vacuities. In this portion lymphocytes, leucocytes and phagocytes are very common. Decaying remnants of fibres may also be found, and also degenerating megacaryocytes and other large cells. Large blood sacs are present in this kidney. Some of them contain only red blood corpuscles, others leucocytes as well. Some have a communication with the venous sinuses, others lack this communication. Six other kidneys of 28 cm animals are at the beginning or at the very end of the activity period and all the others are quite fibrous.

Head kidneys of 29 cm specimens.

Of 21 specimens there are five (24 %) with haemopoietic activity in the kidneys. The other 16 kidneys are quite fibrous. Of these five, two are at the beginning, one at the height and two at the end of an activity period. In one of these kidneys a condition is met with which is rather common in older specimens, viz. a very densely fibrous central mass in which a few haemocytoblasts are distributed. This kidney is apparently regenerating its fibre tissue simultaneously with the ending of a period of activity. The fate of such a dense fibrous mass may be the same as that described in the 32 cm specimens. Blood sacs with macrophages were found in three kidneys.

Head kidneys of 30 cm specimens.

Out of 39 specimens of this class six (15 %) have head kidneys with haemopoietic activity. In kidney 30.1 (Fig. 67) the foremost portion has a central mass with large vacuities, in which the fibrous network is more or less destroyed, and containing a large number of blood cells of every description scattered in the vacuities. The rest of the kidney is fibrous throughout with smaller vacuities lying centrally and without large blood elements in its parts. This part of the kidney seems to be preparing for an activity period. In another kidney (30.4) the activity is nearing its height. The central mass is very spongious, with many blood cells in its vacuities. Kidney 30.5 is of a great interest. It is, like two others, nearing the end of a period. The sections are strongly stained in acid (fuchsin) and show that the spongious central portion of the central mass contains a large number of phagocytes containing fuchsinophilous granules and big elliptical bodies. After the shedding of the contents of the kidneys their central bodies are more or less in tatters (Fig. 68). Blood sacs with macrophages are common. In one kidney of this class (30.2) (Fig. 69) great masses of decaying fibres are collected in a central vacuity,

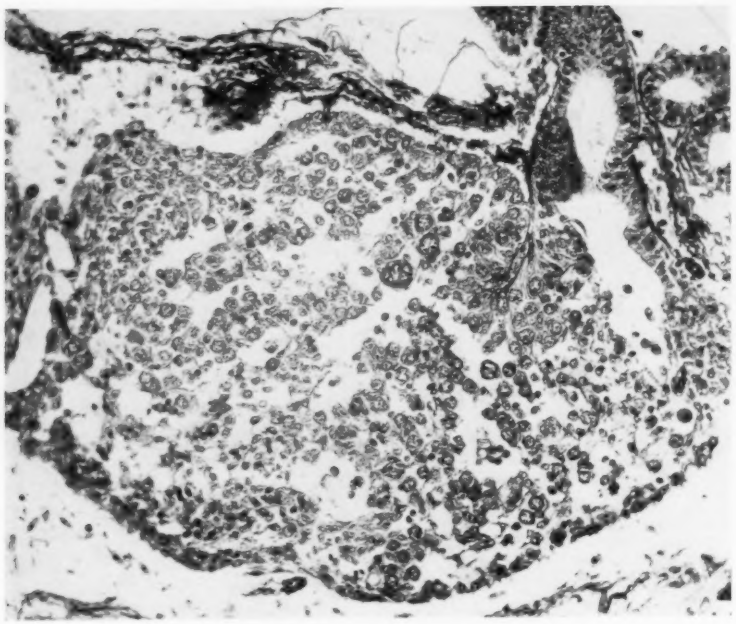


Fig. 67. Pronephros of a 30 cm specimen (30.1).

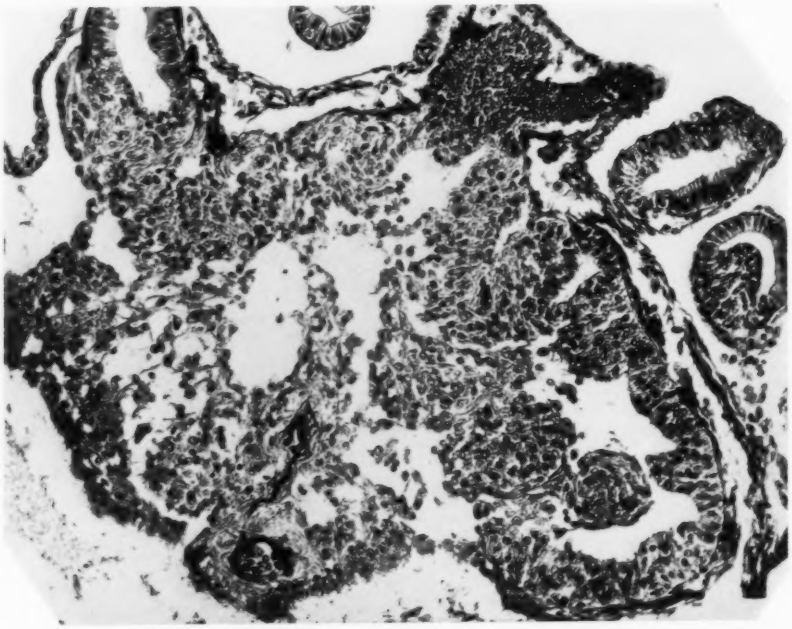


Fig. 68. Pronephros of a 30 cm specimen (30.2). Central mass after delivering its contents. Fibrous type.

VOL.
31
1950

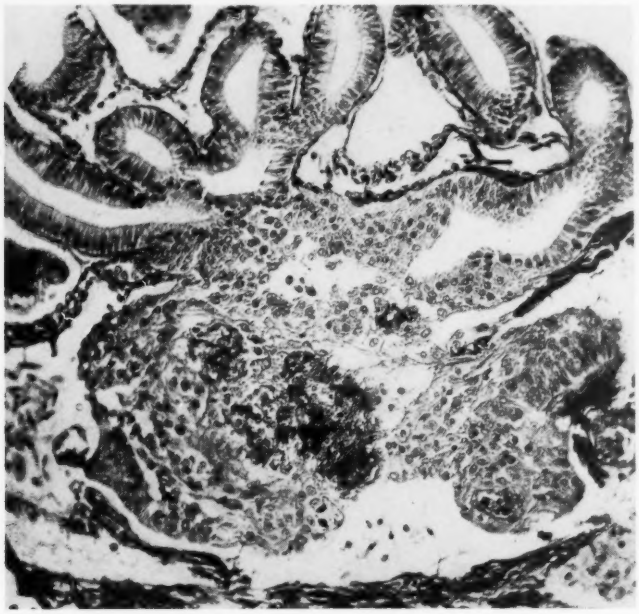


Fig. 69. Pronephros of a 30 cm specimen (30.2). Large detritus masses (fibrin) in the central mass.

which extends to the inner surface of the central mass, where this waste material is discharged into the pronephric vein. Similar matter also may be found in the central cavity.

Among the kidneys showing great haemopoietic activity one is of very peculiar appearance. The anterior part of this kidney is preparing for a new period of activity with a lively increase of the fibre cells. The tubules are slender and not very long, somewhat branched with rather broad central cavities at the surface of the narrow, dense fibrous central mass. This part seems to have recently regenerated. The posterior part of the same kidney is a very big bulky mass of large healthy and decaying haemocytoblasts (Fig. 70). The former forms a thick mantle round the central core where the cells are decaying, and where numerous phagocytes are at work. The big mass is traversed by collagen strands, which seem to form a wide network, in the meshes of which the haemocytoblasts (and other blood elements) are enclosed, often in tube-like groups. The tubules in this part of the kidney are few and pressed together between the central mass and the pericardium. They are slender, and their inner ends open into very insignificant central cavities. Some tubules are completely embedded in the central mass. These tubules are closed at both ends. They thus form tube-like alveoles in the central mass. The swollen part of the central mass has partly incorporated the glomus of the kidney.

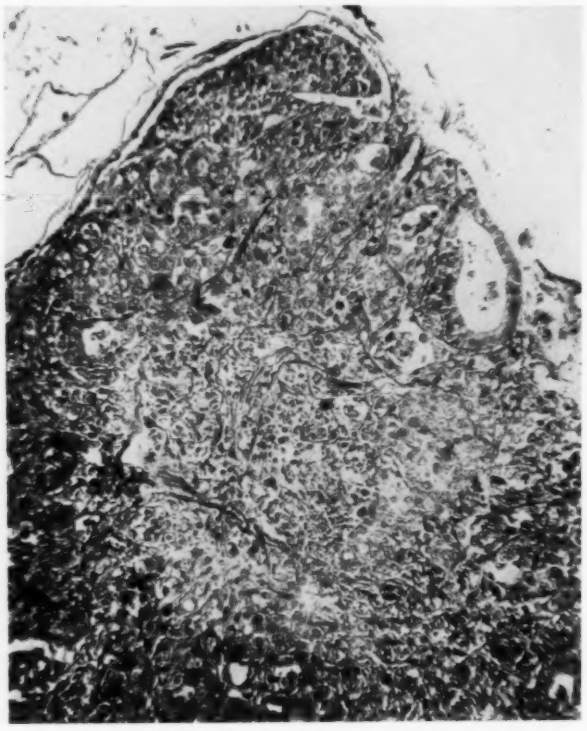


Fig. 70. Pronephros of a 30 cm specimen. Blood elements contained in a collagenous reticulum, developed in connection with trabecles. In the middle a field with degenerating cells.

How this kidney has once arisen is not possible to say with any certainty. It seems, however, that its original tubules may have been almost completely transformed into haemocytoblasts, which thus form the big central mass in a way similar to that described in stages 10.9, 12.10, 13.3, 13.4 and 23. The small weakly developed tubules may be a new set of tubules developing from the mouthpieces of the reduced original tubuli or from alveoles in the central mass. As the tubules of the already regenerated anterior part of this kidney agree with those in the active part, this conclusion seems to be acceptable. When the active part of the kidney sends out its contents into the pronephric vein the degenerating masses in the centre of the central mass come free in the blood, and a big central cavity arises. It is possible, however, that the healthy haemocytoblasts and their derivatives are simultaneously poured into the vein. What is then left of the central mass will probably be a string of regenerating fibre cells at the bases of the small tubules on the surface of the mass. From this string a new central mass probably will arise. The conditions in the regenerating

anterior part of this kidney seem to favour this conception of the future fate of the posterior part of the kidney.

In some of the fibrous kidneys blood sacs are developed in the central mass. In one case, however, a big blood sac was situated on a trabecle, where it traversed the pronephric vein. This sac was thus surrounded by the blood of this vein. The sac contained, besides the usual degenerating lymphocytes, red blood corpuscles and large macrophages, laden with granules from decaying lymphocytes. The sac had a narrow communication with the blood sinuses between the bases of two tubules. It is possible that the sac has obtained its contents through this communication. The sac will probably burst and pour out its cell mass into the pronephric vein.

Head kidneys of 31 cm specimens.

There are 34 specimens of this size in my material. Six of them (13 %) show at least some haemopoietic activity. In one of these six the anterior and middle parts are in activity, numerous haemocytoblasts developing in the tubules. Another specimen is closely reminiscent of the 30 cm specimen of the preceding paragraph, as at least the posterior part of the central mass is very thick completely filling up the pronephric vein. But in contrast to the 30 cm specimen, where the anterior part of the kidney was degenerating, the anterior part of the 31 cm kidney is also strongly developed. The posterior part is filled with big blood cells (haemocytoblasts and their descendants). In the middle of this cell mass inside the tubules the tissue is loosened, but not decaying as in the 30 cm specimen. Phagocytes are present here. The loosening of the central part of the central mass has advanced further in another kidney. In that case large vacuities have been formed. In the vacuities a great amount of phagocytes together with leuco- and lymphocytes as well as haemocytoblasts are present. Otherwise haemocytoblasts and their derivatives are present in the fibrous tissue outside the vacuities. In many fibrous kidneys this process has advanced further as the central vacuity contains only phagocytes, leucocytes and lymphocytes, haemocytoblasts being absent. In this case regeneration of the fibre tissue occurs in the surroundings of the basal parts of the tubuli. When regeneration has advanced some more the original parts of the mass have disappeared and a new mass has arisen from the basal parts of the tubules. Such kidneys are common in my material.

Blood sacs are sometimes present.

Head kidneys of 32 cm specimens.

Out of 35 kidneys only three (8 %) are active. One of these (32.1) has a very compact fibrous central mass with numerous haemocytoblasts between the fibre bundles and along its margin (Fig. 71). The central cavities are very broad, with many tubules opening into them. The external wall of the infundi-

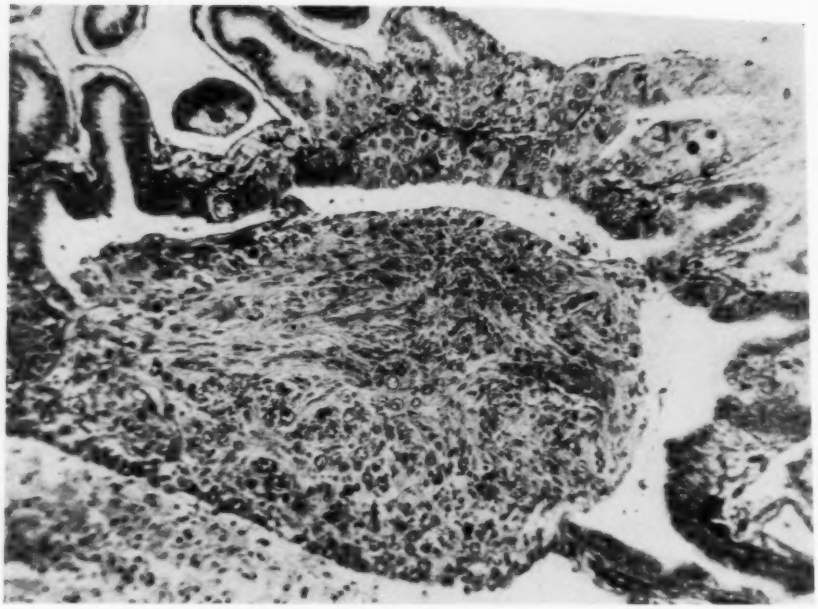


Fig. 71. Central mass and three central cavities of a pronephros of a 32 cm specimen (32.1).

bula and some tubules contain very many haemocytoblasts. At the inner wall of the central cavity and in the central mass some phagocytes are present. The fate of such a compact portion of the central mass is understood when one examines another kidney (32 cm specimen), in which such a mass is present. Here the centre of the mass is degenerating. The fibres are decaying and big phagocytes are working among the degenerating fibres. After such a degeneration not very much will be left of the compact mass, perhaps only small marginal parts, from which regeneration of fibre cells can take place and an invasion of haemocytoblasts into the central cavity can begin.

In a 36 cm specimen the decay of such a compact part of the central mass is almost complete. The fibres are breaking up into granules. In the free spaces in the decaying mass phagocytes and lymphocytes are accumulated.

The interpretation of another kidney of a 32 cm animal is difficult. In this kidney big vacuities are present in the central mass. These spaces contain many phagocytes. The cavities are traversed by considerable strands of fibres. Haemocytoblasts are arranged especially along the trabecles running out from between two tubules and attaching themselves to the wall of the pronephric vein. Groups of haemocytoblasts are entering the cavities. This type of kidney can be explained as having arisen from a kidney that has ended its degeneration

period and has now begun to regenerate its haemocytoblastic contents from cells connected with the trabecles.

The third active kidney is at the very end of an active period. Only a few haemocytoblasts are present. The phagocytosis in it is very pronounced. All the other kidneys of the 32 cm class are fibrous, generally with a very spongious central mass and many phagocytes. In some the central mass is very reduced, tattered. The pronephric vein may then contain masses of decaying lymphocytes as well as numerous phagocytes. Similar masses containing also red blood corpuscles were, moreover, found in some central vacuities. Therefore the decaying masses in the pronephric vein were apparently found originally in the central mass or in blood sacs and thence delivered to the blood. Alveoles and blood sacs are common phenomena in the 32 cm specimens.

Two kidneys belonging to the various specimens differ from the other fibrous kidneys in the behaviour of their central masses, which is fairly voluminous in both of them, filling up the pronephric vein. They may be described as central masses in which a very big cavity has been completely filled with phagocytes so closely packed together that the lumen of the cavity has mostly disappeared. The phagocytes are fairly large. Their cytoplasma contains numerous granules. In the centre of this phagocytic mass there are small spaces without phagocytes or in which the latter are more widely dispersed. It seems possible that these kidneys belong to a type of kidneys in which whole tubules "explode," forming blood elements as described in the preceding pages (Stad. 10, 12, 13, 23, 30). In the present case the columnar cells of the tubules, instead of forming haemocytoblasts, have through successive divisions produced phagocytes. This is a suggestion based upon the fact that in one of these kidneys parts of the columnar epithelium consist of phagocytes. In the phagocytic mass of the central mass peculiar brown-coloured, densely granulated cells are thinly dispersed. They are highly characteristic. Such cells were also found in the metamorphosed parts of the tubuli among their phagocytes. In some parts of the tubules the masses of phagocytes have caused the disappearance of the lumen. It is possible that thin strands of fibres can be present in the central mass of these kidneys, but they are not distinct enough in my sections to allow of a closer examination.

Head kidneys of 33 cm specimens.

Out of 42 kidneys of this class only eight (8 %) are haemopoietically active in some way. Two of these eight kidneys seem to be at the height of their activity. In one of these two the central mass consists practically of one single, nearly spherical dense body on which numerous tubules are implanted with very wide funnels. This body belongs to the anterior part of the kidney (Fig. 72). The small posterior part is fibrous. The most conspicuous elements of the dense body are the haemocytoblasts and their descendants. The centre

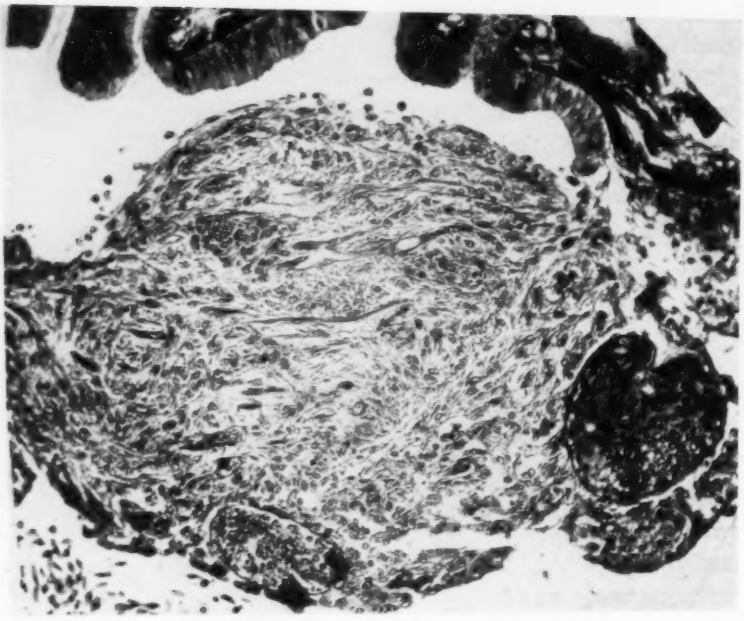


Fig. 72. Central mass of a 33 cm specimen (33.8). Fibres degenerating.

of the body is less densely arranged, with vacuities in which numerous phagocytes are present. A peculiar thing about this kidney is that one of the tubuli has apparently developed a central mass which bulges out in the pericardial cavity instead of in the pronephric vein. This mass consists of a large number of haemocytoblasts and contains a central vacuity with phagocytes.

The other of the two most highly developed kidneys has a central mass like a thick cord extending throughout the whole kidney. This cord is, however, thicker posteriorly than in front. Otherwise this kidney behaves much like the preceding one.

In two other kidneys the activity is also quite lively, but the number of haemocytoblasts is not so high as in the preceding ones. The centre of the central mass contains big vacuities with phagocytes. Four of the active kidneys are at the end of their period of activity, involving the extensive destruction of the central portions of central masses and the regeneration of fibre elements at the external surface at the base of the tubules. In one specimen the central mass is almost reduced to a membrane round the wide central cavities (Fig. 73). Fibre cells and some blood elements are forming at the base of some of the tubuli. This kidney is regenerating. All other kidneys are quite fibrous. Phagocytic activity is strong and the destruction of fibre is a common feature of the

Fig. 73. Central mass of a 33 cm specimen (33.2). Central mass in tatters.

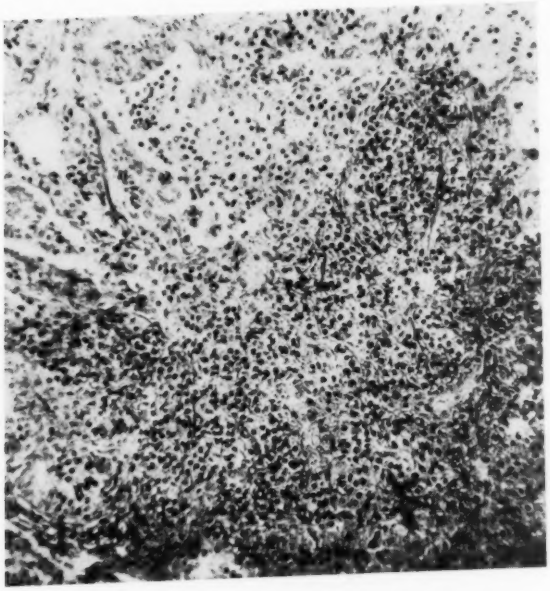


Fig. 74. Central mass of 34 cm specimen; filled up with erythrobasts and their developmental stages. The black spots are nuclei of red blood corpuscles.

central mass. Alveoles are common and blood sacs of different sizes and contents are nearly always present.

Head kidneys of 34 cm specimens.

Of 36 specimens eight (22 %) exhibit at least some haemopoietic activity, but only a single kidney is at height of its activity. This kidney is, however, very peculiar (Fig. 74), being of a type not met with in any other specimen. The central mass is very voluminous, filled to bursting with red blood corpuscles enclosed by thin collagen lamellae in lobules or capsules, many of which also contain haemopoietic cells. The latter are proerythroblasts and polychromatic erythroblasts and normoblasts. The erythrocytes must have been formed in the central mass from such cells. Lymphocytes and some phagocytes were found in small vacuities. The main lumen of the pronephric vein is very narrow, containing but few blood corpuscles, whereas the venous space surrounding the tubules is full of such cells. The tubules are short, their infundibula are wider than might be expected with such short tubuli. The infundibula contain many red blood corpuscles together with some lymphocytes and phagocytes, of which some big ones contain well defined darkstained granulae. In two other kidneys the thick central mass is spongious all through. Proerythrocytes and polychromatic erythroblasts, as well as normoblasts and erythrocytes, are scattered in the vacuities. Phagocytes are plentiful. These kidneys are near the end of a period of activity. The other five active kidneys are at the very end of a period, with strongly regenerating fibrous tissue. All other kidneys of the 34 cm class are fibrous throughout. Blood sacs and epithelial alveoles are common. One of the fibrous kidneys is peculiar, as one of the compartments of its central mass contains a single mass of empty, very thin-walled vacuities enveloped by a thin layer of fibres and fibre cells. The walls of the vacuoles contain small nuclei. Many fibre cells also are present in the alveolar mass. In preceding pages I have described such central masses, where the tissue contained phagocytes between which thin membranes were diffusely discernible. I presume that the kidney in question belongs to this latter type. This presumption is based upon the following observation. In another kidney (34 cm stage) (Fig. 75) belonging to the same type as the other two, the external part of the central mass consists chiefly of densely crowded phagocytes, the cell membranes of which are pressed against each other forming a thin, easily discernible membrane. The cytoplasma of the cells contains granules in all parts. Among such cells are others of the same shape but with the granules around or close to the nucleus. The granula-free part of the cell body is occupied by a great vacuole. In the main part of the central mass the cytoplasma of the cells and the nucleus are pushed to the surface of the cell, the rest being occupied by a great vacuole. The further fate of such a central mass could not be studied accurately in my material, but it seems as if the regenerating fibre cells at the

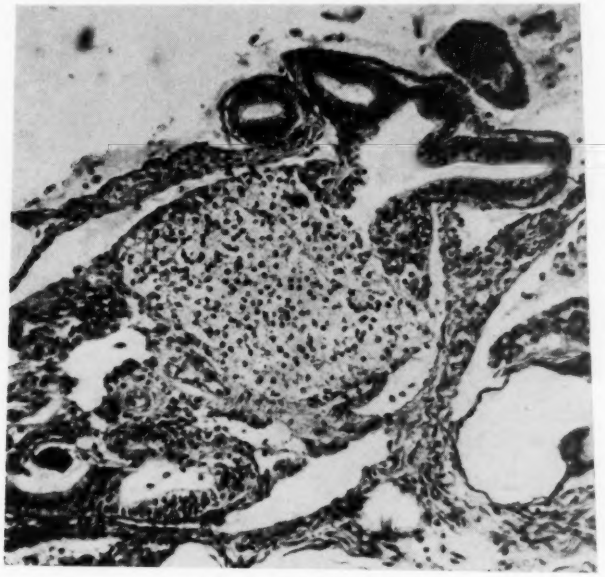


Fig. 75. Central cavity and vacuity filled with phagocytes in a 34 cm specimen.

bases of the tubules had something to do with the disappearance of these degenerated phagocytes, big strands of fibres growing into the phagocytic mass.

Among the fibrous kidneys there are two main types present. In the first type the central mass is rather thick. Every compartment of it is more or less decaying in its central part, filled with phagocytes, remnants of fibre cells, lympho- and leucocytes. In some of the kidneys of this type the contents are poured out into the pronephric vein. Regeneration of fibre cells is proceeding from the basal part of the tubules and from the spaces between the tubules. In the second type the central mass is slender, forming a string connecting the regenerating fibre masses at the bases of the tubules. This type of kidney arises after the contents of a thick central mass have been destroyed. The second type is a regenerating type. These two types of fibrous kidneys are generally found in all kidneys of specimens above 34 cm body length.

Head kidneys of 35 cm specimens.

Out of 43 kidneys of this class ten (23 %) exhibit at least some haemocytoblasts and have therefore been classified as kidneys with haemopoietic activity. All the others are fibrous.

In none of the specimens does the whole central mass contain haemocytoblasts. They are only present in some part of it, mostly in the anterior part. One of these ten kidneys is at the end of a phagocytic period, the main part of

the central mass having been consumed and new fibre cells being formed at the bases of the tubules. In one of the compartments haemocytoblasts are forming between the newly developed fibre cells. In another kidney the anterior part of the central mass is newly regenerated. It has a very broad infundibulum belonging to a system of tubules. The central cavity contains only a few phagocytes. In the tubules belonging to this portion the basal parts practically consist of haemocytoblasts and the wide central cavity is also lined with such cells. The central mass contains a dense mass of haemocytoblasts and their descendants. A large number of megacaryocytes of different sizes is present. They may contain a varying number of nuclei, from one single large one to many small ones. Such small nuclei cannot be distinguished from the nuclei of lymphocytes and it seems possible that these nuclei may be set free together with a small plasmatic portion, forming lymphocytes. Blood sacs and alveoles are common. The other active kidneys are at the end of theis period of haemopoietic activity involving the great destruction of their central masses or the formation of new central masses. The fibrous kidneys are like those in the preceding stage. In the 35 cm stage also central masses are present entirely filled with densely packed phagocytes, as described in the 34 cm stage. A common feature of all these kidneys is the extensive phagocytosis. The phagocytes are very variable as to size and contents. Large phagocytes containing big ovoid dark-stained bodies ("yellow bodies") were found in some cases.

VOL. 31 1950

Head kidneys of 36 cm specimens.

Out of 33 kidneys of this class 5 (19 %) are in activity with at least some haemocytoblasts. In one of these kidneys the medium portion is in high haemopoietic activity. The central cavities are small but the central mass contains a big central vacuity. This cavity is about to be filled with haemocytoblasts developing at the bases of the tubules and in the vicinity of the trabecles. Many of the haemocytoblasts are dividing, producing medium-sized and small mononuclears. Megacaryocytes of different sizes with a variable number of nuclei are common. Phagocytes are numerous. The other parts of the central mass of this kidney contain large vacuities with many phagocytes, but there are no large cells in the tissue surrounding them. Masses of degenerating cells are found in the central cavity and in some of the tubules. These masses contain red blood corpuscles and lymphocytes embedded in a fibrinous mass (coagulated blood). Some of the central cavities are widening through alveoles being added to the borders of the infundibula. Another kidney is like the preceding, but the activity is not quite so strong. The central cavities, however, have joined the central vacuities, which contain numerous phagocytes, and also some lymphocytes and leucocytes and medium-sized more or less free mononuclears (proerythroblasts). At some places such cells are delivered to the blood stream. Megacaryocytes are rare. In a third kidney, in which the

central mass is largely degenerating or discomposing, there is at its posterior end a small centre of haemocytoblasts at the bases of the tubuli. In the vacuities and the central cavities many big phagocytes are present containing dark granulae. In large masses of decomposing fibres phagocytes are present. Such cells may form quite large fields in the tissue. In some of the phagocytes are found ovoid bodies, as in the 35 cm stage. In the present stage they are yellow, the iron-haemotoxylin being strongly extracted. The fourth and fifth kidneys of this class have very reduced central masses, with developing fibre cells at the bases of the tubuli and around strong trabecles. A few haemocytoblasts are present among the fibre cells.

The other kidneys are in different stages of fibre development or destruction, either with a very reduced central mass and developing new fibres or with a rather thick central mass with vacuities and a more or less abundant supply of phagocytes and degenerating fibre cells. Blood sacs are very common. Some of them are very large. In one case a blood sac fills up almost the entire central mass.

Head kidneys of 37 cm specimens.

Out of 21 kidneys of this class three (14 %) show haemopoietic activity. They have a slender central mass, with hemocytoblasts only in connection with a few tubuli at the anterior end or in the middle portion of the kidney. A third kidney has a weakly developed central mass, which, however, contains large central cavities with many phagocytes. In this kidney a few middle-sized mononuclears are situated at the surface of the central mass. All the other kidneys of this class are fibrous. Phagocytic activity is very conspicuous.

Head kidneys of 38 cm specimens.

Of 19 specimens only one (5 %) has any haemocytoblasts. They are located at the posterior end of the weakly developed central mass. The remaining kidneys of this class are fibrous, with phagocytes.

Head kidneys of 39 cm specimens.

There are 11 kidneys of this class present. One of them (9 %) contains a few medium-sized mononuclears and haemocytoblasts among the short fibre cells between the bases of the tubuli. This kidney is apparently regenerating, its fibre cells preparing for fresh activity. The remaining kidneys are fibrous, with more or less advanced phagocytic activity.

Head kidneys of 40 cm specimens.

The ten kidneys of this class at my disposal are all fibrous with large number of phagocytes, containing enclosed bodies of different kinds.

Head kidneys of 41—46 cm specimens.

The five kidneys of these classes are all fibrous, with distinct phagocytic activity.

THE MORPHOLOGICAL ASPECTS OF THE FUNCTIONAL DEVELOPMENT OF THE HEAD KIDNEY.

In the preceding pages the morphology of the different head kidneys contained in my material has been outlined. The variety of the pictures resulting from my investigations, make a very bewildering impression and it seems often impossible to bring the different head kidneys into relation to one another. Nevertheless. I shall try to give a sketch of the morphological aspects of the functional development of the head kidney, as they seem to result from the preceding descriptions.

As our starting-point we shall select a young fibrous kidney. This consists of a number of tubules with closed central cavities dipping into the quite fibrous central mass. The latter forms a string. The fibre cells are interwoven so as to form a dense meshwork, in which lymphocytes or young phagocytes are enclosed. The central cavities are small. Some of its epithelial-like cells are pushed into the cavity, where they form a set of small phagocytes. As the pericardial fluid is driven through the activity of the ciliar apparatus of the columnar cells of the tubules into the cavity, the latter expands and the epithelium at the bottom of it becomes flattened so that it ultimately bursts. Then the vacuities of the fibrous tissue become connected with the central cavity and a way through the central mass is established. This is, however, not an open way but merely a way that leads from one vacuity to another. The pericardial fluid on its way through the kidney at first meets a number of phagocytes, which are engaged in cleaning the fluid from particles contained in it. Then the fluid becomes filtered through a fibrous filter, in the meshes of which it has to pass a set of other phagocytes. At last the fluid, probably more or less freed from certain kinds of matter, is poured out into the pronephric vein.

In the fibrous state the head kidney apparently works as a filter, retaining certain kinds of matter contained in the pericardial fluid. In older kidneys these forms of matter become very conspicuous as granulae or cristalloids, in younger ones they are represented by a fine granulation of the cytoplasm. As the excretory products of the glomus are poured out into the pericardial pocket, which is connected with the central mass by one or two tubules, part of these products must undoubtedly pass through the central mass, there to be filtered. In view of the fact that in older animals the phagocytes may contain the same

VOL. 31 1950

dark excretory bodies that are characteristic of the epithelium of the mesonephric duct, they may be excretory products in the pronephros also.

The pronephros of the fibrous type must thus be considered to have an *excretory function* as well as the *function of conducting pericardial fluid to the pronephric vein.*

The fibrous stage of the head kidney is followed by the lymphoid or haemopoietic stage. This is initiated by the development of haemocytoblasts from columnar cells in the tubules or in the infundibulum and its surroundings. These developing cells generally cause a swelling of the central mass, which ultimately becomes so bulky that it fills up the pronephric vein almost completely, cutting down the blood stream. As the blood elements increase in the central mass, the vacuoles in the fibrous tissue are widened, probably through the destructive work of the phagocytes, the mass loses some of its property as a filter and the pericardial fluid can stream through the central mass more rapidly than before. However, in a central mass of this description the phagocytes are more numerous than before partly in the central cavity, partly in the vacuities, a fact that may compensate for the rapidity of the stream of the pericardial fluid. Later, as the blood-forming tissue completely fills up the central mass, it seems very probable that the stream of fluid ceases altogether or at least becomes very slow. Now the phagocytes are more widely scattered among the blood-forming cells or else have disappeared. By this time the kidney should have lost its property of being excretory if the blood-forming activity were uniform throughout the entire kidney. But generally the development of some part of the kidney is arrested at the fibrous stage and this part can then fulfil the excretory function.

During the "lymphatic" period the blood elements increase very rapidly through divisions of the various larger cells, and consequently the central mass swells considerably. The fibrous lining layer grows thinner and at last bursts or is disolved. Then the contents of the central mass are poured out into the pronephric vein. The bursting of the membranes and the outstreaming of the contents of the central mass apparently take place under pressure from the fluid that is driven into the central mass through the ciliar activity of the tubules, as the stream of the blood elements seems to be so strong that the fibre capsules of the blood cells burst open and leave the mass in tatters.

After the expulsion of the blood cells the central mass is very spongious, with large vacuities in which destroyed parts of fibre cells are attacked by phagocytes. The lining membrane of the central mass more or less resumes its original appearance. Blood elements previously lying in or near this membrane are left in it and blood cells are likewise left in the walls between the compartments. The central mass has also resumed its slender shape.

However, already before the bursting of the central mass a regeneration of the fibrous tissue has begun in the surroundings of the infundibular and at

the bases of the tubules. New fibrous tissue is here developing from columnar cells of the tubules. These cells successively grow out, forming a new set of fibrous tissue substituting the older one that was destroyed by the bursting of the central mass. The old lining membrane of the central mass disappears and the new fibrous tissue forms a new membrane. Now the kidney is back at its starting-point and has reached a new lymphatic period.

During this development of the central mass the tubules have undergone a change in length. At the beginning of the period the tubules are comparatively long, in older specimens branched. When haemocytoblasts are developing in the basal parts of the tubules, this part gradually shortens, as the haemocytoblasts are transferred to the central mass. As soon as the basal part has shortened to a branching point of the tube the branch becomes an opening in the central cavity, belonging to the tubule of which it is a branch. Simultaneously the tubules become shortened and the central cavity wider. After the "lymphatic" activity reached its climax, the tubules lengthen again.

In the preceding pages the "normal" type of development of the pronephros has been sketched. But besides this type I have found three others, in which the "lymphoid" activity has assumed other aspects. As these other types are comparatively rare they could not be investigated in detail.

These three types have this in common, that the formation of blood elements in some of the tubuli is very abundant. In some of them practically all the columnar cells have become transformed into haemocytoblasts and their descendants before they leave the tubule. In two 13 cm specimens this development has caused the complete obliteration of the canal in some of the tubules. Such tubules are directly incorporated with the corresponding central mass. In these two kidneys the basal part of the tubules contain only cells of smaller sizes than the haemocytoblasts. There thus are no haemocytoblasts in the central mass.

In a specimen of 13 cm body length practically all the columnar cells of the tubule, excepting those of the mouthpiece, are haemocytoblasts, which completely fill up the canal. In this case the central mass is filled with a very dense mass of haemocytoblasts. In this mass a central cavity arise through degeneration of the central part of the mass of haemocytoblasts. As in this case all the tubules of the posterior half of the kidney are transformed into large sacs containing haemocytoblasts, these are separated from each other by collagenous membranes, resulting from the collagenous membranes of the original tubes. As already mentioned, the mouthpieces of such transformed tubuli remain unchanged. In another specimen a regeneration of tubules has begun from such mouthpieces and small slender tubules arise from the very voluminous central mass. When ultimately the central mass bursts, there will only remain these tubules and a large number of regenerating fibre cells at their bases. The anterior part of the pronephros is of that very reduced type.

VOL. 31 1950

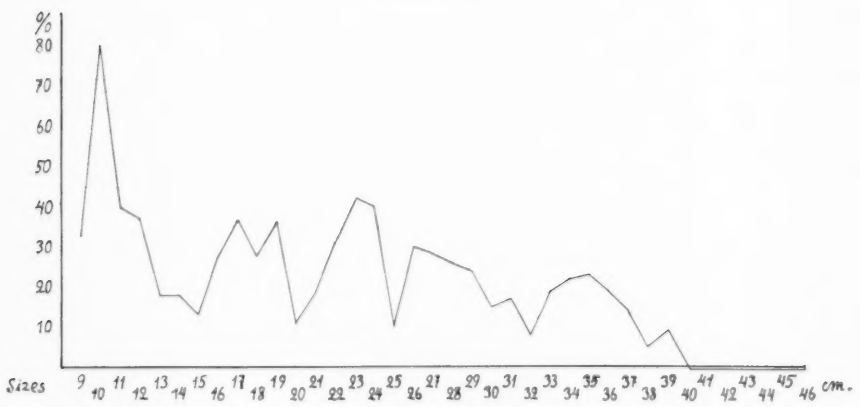
In another case the development is similar to the preceding one, but here the columnar cells have all been replaced by a dense small-celled mass, which fills up the lumen of the tubule and causes it to swell up into a sac. This mass also forms part of the central mass. In some cases compartments of the central mass contain such a cell mass, which in places begins to disintegrate, its cells becoming free from one another. In such cases it is to recognize the free cells as phagocytes. The haemopoietic activity in these compartments of the pronephros is thus restricted to the production of phagocytes.

The role played by the phagocytes during the normal functional development is partly that of cleaning the pericardial fluid from certain kinds of matter during the filtration process, partly storing up the material from decaying fibres and cells after the delivery of the blood elements to the pronephric vein. Ultimately, however, the phagocytes also enter the vein. It seems that the phagocytes have already dissolved their more solid enclosures before entering the blood-stream, so that their vacuoles only contain a fluid. This conclusion is partly based on the fact that in blood smears not a single phagocyte was found containing such solid bodies.

PERIODICAL ACTIVITY OF THE HEAD KIDNEY.

In the annexed table the material upon which the preceding summary description of my material is based is analysed with reference to the occurrance of haemopoietic activity. The material is somewhat inadequate in regard to the number of specimens in the different size groups but nevertheless it gives some idea of the distribution of the haemopoietic active sizes of animals,

TABLE I.



Size of specimen cm	Total number	Inactive kidneys (fibrous)		Active kidneys (lymphoid)	
		Number	% of total	Number	% of total
9	3	2	66	1	33
10	15	3	20	12	80
11	27	16	60	11	40
12	68	43	75	25	37
13	60	49	82	11	18
14	43	36	82	7	18
15	47	41	87	6	13
16	76	55	72	21	28
17	32	20	63	12	37
18	29	21	72	8	28
19	30	19	64	11	36
20	27	24	89	3	11
21	21	17	81	4	19
22	16	11	69	5	31
23	22	12	58	10	42
24	20	12	60	8	40
25	31	28	90	3	10
26	20	14	70	6	30
27	25	18	72	7	28
28	31	23	74	8	26
29	21	16	76	5	24
30	39	33	85	6	15
31	34	28	83	6	17
32	35	32	92	3	8
33	42	34	81	8	19
34	36	28	78	8	22
35	43	33	77	10	23
36	33	28	81	5	19
37	21	18	86	3	14
38	19	18	95	1	5
39	11	10	91	1	9
40	10	10	100	—	0
41—46	6	6	100	—	0
	993	758	76.3	235	23.7

especially if the percentage figures are compared. The annexed curve (Table), which is based upon these figures, gives a fairly clear idea of this distribution. In the first half of this curve there are three distinct maxima of haemopoietic activity, viz. at 10, 17—19 and 23—24 cm body lengths. In the second half the maxima are somewhat indistinct, the curve describing two long waves, with maxima at 26—27 and 34—35 cm body lengths. The haemopoietic activity then ceases at 40 cm. How is the difference between the first and second halves of the curve to be explained? No other explanation seems possible than

VOL. 31 1950

that the function of the head kidney alters with increasing body length (age). In the earlier stages of its development the head kidney is an important haemopoietic organ, whereas in the later stages it has more or less lost its haemopoietic importance and has become a phagocytic organ. In the 26—28 cm stages the phagocytic activity has already increased to a marked extent and the haemopoietic activity has decreased; in the still later stages this is very obvious. From the 40 cm stage onwards the haemopoietic activity has ceased altogether.

The figures show that in the material consisting of 993 kidneys only about 23, 7 % are in some phase of haemopoietic activity. The other 76, 3 % belong to fibrous kidneys without haemopoietic activity. This fact seems to indicate that haemopoietic activity is not the chief function of the kidney, but that the excretory function, which is largely based upon the activity of the phagocytes especially in the fibrous stages, is of greater importance.

A SHORT SUMMARY.

This study is based upon serial sections of about 1 000 (993) head kidneys of *Myxine* specimens of different sizes ranging from 9.3 cm to 46 cm body length and upon a large number of blood smears.

The kidneys were fixed in Smith's, Flemming's, Helly's, Maximov's, Bouins's and others' fluids. Staining was performed in Heidenbrain's iron-haematoxylin with contrastaining in picro-fuchsin (Hansen) and for haematological purposes according to the methods of Giemsa, May-Grünewald, Pappenheim, Wright and others.

1) The head kidney consists of two parts: the pronephros proper and the pronephric glomus (Malpighian body).

2) The pronephros proper consists of the tubules and the central mass.

3) The tubules are ciliated tubes, built up of a columnar epithelium. They begin with a nephrostome in the pericardial wall and end in the central mass with a central cavity. They are basally surrounded with thick rings of collagenous tissue, which are connected with the wall of the pronephric vein by collagenous bands (trabeculae), piercing the central mass.

4) The central mass is very variable as to size. It hangs in the tubules but otherwise floats free in the pronephric vein, *without* any endothelial covering.

5) The central mass is built up of the fibrous tissue, consisting of fibre cells, developing from the columnar cells of the tubules. The fibre cells form an alveolar reticulum, in the meshes of which different kinds of blood elements are enclosed.

6) Blood elements develop either from columnar cells of the tubules or from fibre cells.

7) Erythrocytes, granulocytes, lymphocytes, spindle cells and phagocytes originate in the head kidney, and their developmental stages are found in it. The quantity of these blood corpuscles in the head kidney is not sufficient for supplying the whole body with its needs in the way of blood corpuscles. Other sources for the formation of blood were found in the liver and in the spleen, but there may be other hitherto unknown blood-forming organs in the body of *Myxine*.

8) Besides the lymphatic organs of the intestinal folds a spleen was sometimes found in the anterior end of the intestine.

9) The red blood corpuscles pass, as in other vertebrates, through the following developmental stages: haemocytoblasts, proerythroblasts, polychromatic erythroblasts and normoblasts. The last-mentioned become transformed into erythrocytes.

10) From the polychromatic erythroblasts granulocytes may also develop. It could not be decided whether or not these cells have a developmental series of their own.

11) Lymphocytes and phagocytes develop direct from different sources: columnar cells, fibre cells or excretory cells of the glomus. Spindle cells are descendants of fibre cells.

12) There are two distinct functional phases present in the head kidneys, viz. one quite fibrous, excretory and phagocytotic phase and one haemopoietic. These two phases alternate with one another. Upon a fibrous phase follows a haemopoietic one and again a fibrous. From specimens of 9 cm body length to those of 46 cm there are six fibrous and five haemopoietic phases; of 993 kidneys 750 were fibrous and 235 were haemopoietic. The fibrous thus seem to be the most important. The haemopoietic activity is especially concentrated in animals of 10, 17—19, 23—24, 26—27 and 35 cm body length. This activity decreases from about 26—27 cm body length, while at the same time the phagocytotic activity increases.

13) During the fibrous phase the head kidney acts as a filter, through which fluid from the pericardial cavity is led to the pronephric vein. On its way the fluid has to pass many phagocytes, which take up matter from the fluid and store it up.

14) During the haemopoietic periods the filter function decreases as the haemopoietic increases, but it does not cease altogether as some part of the kidney always seems to keep some of its fibrous character.

15) The blood elements formed in the head kidney are poured out into the pronephric vein and thus come into the circulating blood, where many of them degenerate. This degeneration has been described.

16) As the blood elements are delivered to the circulating blood the central mass is largely destroyed. The decaying parts are more or less consumed by

VOL. 31 1950

phagocytes and are also conveyed to the blood. The central mass then regenerates from fibre cells developing from columnar cells at the base of the tubules.

17) The tubules become shorter during the period of haemopoietic activity. In some cases all their columnar cells are transformed into blood elements. In such cases the tubules are entirely consumed with exception of their mouth-pieces. Regeneration then takes place from the mouth-pieces. The formation of new tubules is described.

18) Small epitheloid alveoles containing a colloid-like mass are found in the central mass. The presence of these may indicate some hormonal activity of the head kidney. Through the activity of the head kidney large masses of cells, cell products, decaying cells, waste material and so forth are delivered to the blood stream. As these products of the kidney may contain matters of hormonal importance, the head kidney *may* also be a hormonal organ of some importance.

19) Blood is supplied to the pronephros by the pronephric vein, in which the central mass swims. In older animals the vein forms a dense plexus around the basal part of the tubules and along their sides. A single artery was followed to the posterior end of the head kidney. Branches of the venous plexus follow the trabecles. Such branches may come inflated to form big blood sacs, in which masses of blood corpuscles are destroyed and consumed by phagocytes.

20) The glomus of the pronephros was studied. The excretory cell of the pronephric glomerulus deliver their products to the pericardial cavity, whence they are transported through the pronephric tubules to the central mass and to the pronephric vein. Large phagocytes were found in the capillary convolute of the glomus.

21) In the mesonephric glomeruli were found large phagocytes identical with those in the pronephric glomus.

LITERATURE CITED.

Conel, J. le Roy (1917). The urogenital system of Myxinoids. Journ. of Morph. Vol 29.

Holmgren, N. (1846). On two embryos of Myxine glutinosa. Acta Zoologica. Bd 27.

Jordan, H. E. and Speidel, C. C. (1930). Blood formation in Cyclostomes. Amer. Journ. of Anat. Vol 46.

Kirkaldy, J. W. (1894). On the head kidney of Myxine. Quart. Journ. Micr. Sci. Vol. 35.

Maas, O. (1897). Über Entwicklungsstadien der Vorniere und Urniere by Myxine. Zool. Jahrb. Alt. Anat. Bd 10.

Martin, G. Lama San (1944). Observationes hematologicas en la especie Bdellostoma Polytrema. Bull. Soc. Biol. Concepción. Tomo 19.

Müller, J. (1843). Untersuchungen über die Eingeweide der Fische. Schluss der vergleichenden Anatomie der Myxinoiden. Abh. König Akad. der Wissens. zu Berlin.

Müller, W. (1875). Über das Urogenitalsystem des Amphioxus und der Cyclostomen Jen. Zeit. f. Naturwiss. Bd 9.

PRICE, G. C. (1896). Development of the Excretory Organs of a Myxinoid, Bdellostoma stouti. Zool. Jahrb. Bd 10. Anat.
— (1904). A further Study of the Development of the Excretory Organs in Bdellostoma stouti. Amer. Journ. Anat. Vol. 4.
— (1910). The Structure and Function of the Adult Head Kidney of Bdellostoma stouti. Journ. exper. Zool. Vol. 9.
SIMON, R. (1896). Das Excretionssystem der Myxinoiden in seiner Bedeutung für die morphologische Auffassung des Urogenitalsystems der Wirbeltiere. Festschr. f. Gegenbaur. Bd 3.
— (1897 a). Das Excretionssystem der Myxinoiden. Anat. Anz. Bd 13.
— (1897 b). Vorniere und Urniere. Anat. Anz. Bd 13.
SPENGEL, J. W. (1897 a). Die Excretionsorgane von Myxine. Anat. Anz. Bd 13.
— (1897 b). Semon's Schilderung des Mesonephros von Myxine. Anat. Anz. Bd 13.
WELDON, W. F. R. (1884). On the head kidney of Bdellostoma, with a suggestion as to the origin of the suprarenal bodies. Quart. Journ. Micr. Sci. Vol. 24.

VOL. 31 1950

ON THE MOBILITY OF ENTIRE ZOIDS IN BRYOZOA

BY

LARS SILÉN

CONTENTS.

1. Introduction 349
2. Avicularia of the Bicellariellidae 350
3. Autozoids of certain Stolonifera 358
4. Autozoids of Kinetoskias 366
5. Relation to ordinary muscular actions of autozoids; stimuli; co-ordination problem 374
6. Summary 382

1. INTRODUCTION.

It has been known for a long time that in the colonies of some few Gymnolaematous Bryozoa, all or some zoids are able to alter their position in relation to the adjacent zoids. The movement is, directly or indirectly, effected by muscular action. Our knowledge of this matter is mainly based on scattered remarks in the literature.—The well-known movements of the zoarium as a whole in *Cristatella* and related Phylactolaematous forms (cf. MARCUS 1926 c) and of the single zoids of *Monobryozoon* (REMANE 1936 and 1938) are caused in quite different ways and do not lie within the scope of the present research.

The following types of zoids claimed to be movable can be discerned: the "bird's head" avicularia of the Bicellariellidae, the autozoids of several Stolonifera, certain kenozoids of *Caulibugula* (Bicellariellidae) and, finally, the autozoids of *Kinetoskias* (Bicellariellidae) and the comparatively closely related *Euoplozoum* (Euoplozoidae).

A great many forms have been examined by the author for the present account. Living material has been studied at the marine stations at Kristineberg (west coast of Sweden) and at Marseille, Plymouth, and Trondheim. Preserved material has been placed at my disposal by the Swedish Museum of Natural History in Stockholm, the Zoological Institute at Uppsala, and the British Museum of Natural History in London. The author is greatly indebted to the curators of the above-mentioned institutes, Dr. G. GUSTAFSON, Kristine-

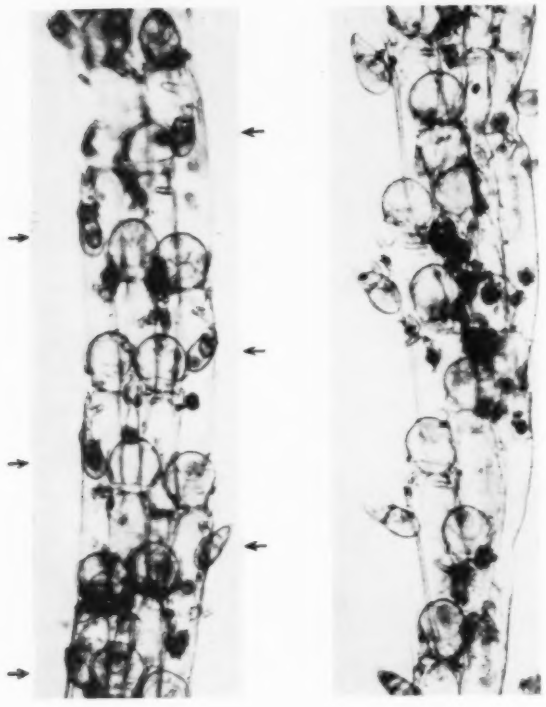


Fig. 1. *Bugula sp.* from Plymouth. Branch in straight (left) and oblique (right) frontal view to show position of avicularia. 35 ×.

VOL. 31 1950

berg, M. le Professeur J.-M. PÉRÈS, Marseille, Dr. J. F. RUSSELL, Plymouth, the late Dr. C. DONS, Trondheim, Professor N. ODHNER, Stockholm, Professor S. HÖRSTADIUS, Uppsala, and Dr. ANNA B. HASTINGS, curator of the Bryozoa collections at the British Museum, for their valuable help.

2. AVICULARIA OF THE BICELLARIELLIDAE.

The avicularia of *Bugula* have attracted the attention of numerous examiners of the Bryozoa since ELLIS's (1756) days. They have a characteristic shape of minute bird's heads resting on ordinary zoids by means of delicate peduncles. This type of avicularium is met with in several other genera of the Bicellariellidae as well but is, in its characteristic form, unknown in the remaining Bryozoa, where the avicularia are firmly attached to the adjacent zoids.

The incessant movements of these avicularia first described by DARWIN (1839, Ch. 9, May 19th) afford a peculiar sight familiar to every student of living Bugulae. They sway to and fro along a fixed course in a striking way.

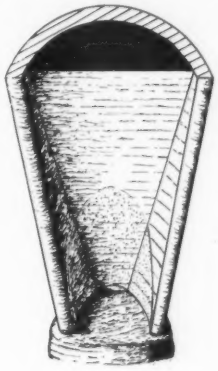


Fig. 2. Diagrammatic picture of avicularian peduncle in frontal view to show attachment to mother zoid, extension of frontal membrane, and extension of chitinous wall (dotted area) in relation to calcified wall.

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Several authors have commented more or less briefly upon this movement. However, two attempts only have been made to explain its mechanism.

CALVET (1900, p. 53, textfig. 8) described muscles running longitudinally within the peduncle of the avicularium of *Bugula sabatieri* Calvet. He was of the opinion that the avicularia of the Bicellariellidae in general are moved by these muscles, one group of fibres—called the flexor muscle—pulling the avicularium forwards and another group—called the extensor muscle—pulling it backwards. The points of origin and insertion of the muscles, however, were not distinguished and the exact details of the mechanism are consequently obscure.

In a previous paper (SILÉN 1938, p. 240) the present author demonstrated that the avicularian peduncle of *Dendrobeania birostrata* (Yanagi & Okada) has no such muscles. Whether this feature is specific to *Dendrobeania* or of more general validity was left to further researches.

MARCUS (1939, p. 202) stated that *Bugula ditrupae* Busk is likewise devoid of the muscles in question. According to him, CALVET has taken threads of the funicular tissue for muscles. He described (cf. also Pl. 17 fig. 42 of the cited work) how in this species the avicularian peduncle emanates from a projection of its mother zoid—a feature common in the Bicellariellidae—and how muscles extend within this projection to the proximal end of the peduncle, muscles that would effect the movements of the avicularium.

According to both CALVET's and MARCUS's theories the avicularium is thus moved by the direct work of two antagonistic muscle bundles. The fundamental difference between them is that according to CALVET's theory the shape of the peduncle must change at every movement, alternately bending first in one and then the other direction, whereas the consequence of MARCUS's theory would be that the avicularium is moved as a whole without any change of shape as the muscles are situated outside the avicularium.

It has been generally assumed that all avicularia of the present type of the Bicellariellidae have the power of moving. This is an inference from the conditions in the comparatively few forms that have been observed in their living state. However, already DARWIN (l.c.) mentioned briefly that in certain (which?) species the bird's head avicularia do not move, and MARCUS (op. cit., p. 201) considers the avicularia of this type in *Beania intermedia* (Hincks) to be immovable as they always hold the same position (the material examined was apparently preserved). The present author can confirm this statement having made the same observation in preserved material of different species of *Beania* and *Dendrobeania* and, what is decisive, in living colonies of *Beania hirtissima* (Heller) at Marseille and of *Dendrobeania murrayana* (Johnst.) at Trondheim, the avicularia and autozoids of which functioned perfectly in other ways and thus were with certainty in full vigour. All of the species, where avicularian movement has been directly seen, belong to the genera *Bugula* (observed by the writer in *B. flabellata* [Thomps.], *B. turbinata* Alder, and *B. sp. 1* at Plymouth [closely related to *B. flabellata*], and in *B. sp. 2* [young colony] at Marseille) and *Bicellariella* (observed by the writer in *B. ciliata* [L.] at Kristineberg). It is now evident that we cannot state anything in the present respect about species not studied alive—though it is possible that all the members of *Bugula* and *Bicellariella* behave in a similar way—and the scope of the present study is thus considerably restricted.

All of the above-mentioned forms of *Bugula* and *Bicellariella*, where the writer has observed avicularian movement, have been sectioned. He has entirely failed to identify any muscle fibres in the peduncles of their avicularia, either in the true peduncles of the avicularium itself as described by CALVET or in the corresponding projection of its mother zoid as described by MARCUS. Strands of funicular tissue, such as MARCUS assumed to have been mistaken by CALVET for muscle fibres, were distinct in both places.

Evidently the movements of the avicularium are the result of some sort of muscle action. It follows from the above that this type of zoid possesses no other muscles than the well-known adductor and abductor. The former is inserted directly into the mandible and thus cannot have any influence on the movements of the entire avicularium. The abductor consequently remains as the sole possibility.

The abductor fibres emanate from the wall of the swollen part of the avicularium and are inserted into the frontal membrane behind the proximal margin of the mandible (fig. 3). The obvious effect of their work is the opening of the mandible: the fibres contract and lower the frontal membrane, which pulls the proximal end of the mandible downwards so that the mandible turns round a traverse hinge, situated somewhat distally to its proximal margin, with the result that the large distal part of the mandible is elevated and swings back (for details cf. SILÉN 1938, pp. 245 ff., etc.).

Already in the cited paper it was mentioned that the abductor of *Dendrobeania* consists of two parts, emanating from the distal and proximal parts of the curved wall respectively and their tendons inserting near each other in the median line of the frontal membrane. The same feature is exhibited by all the members of *Bugula* and *Bicellariella* treated here and is there even more accentuated, there being a small but distinct distance between the insertion points in the frontal membrane (fig. 3).

The short peduncle belonging to the avicularium itself is circular of cross section in *Dendrobeania* and its wall seems to be of equal thickness and structure all round at a certain level (l.c.). Previous descriptions of the corresponding peduncles in *Bugula* or *Bicellariella* have not revealed any particular features differing from that general picture.

However, a close scrutiny of the peduncles in the *Bugula* species (the avicularia of *Bicellariella* proved to be too small for reliable observations in the present respect) gave quite a different picture of their detailed structure (figs. 2, 3).

The back wall of the peduncle is shaped like one half of a longitudinally divided tube, semicircular of cross section. The calcareous layer of the wall reaches down to the fixation area of the peduncle along the disto-lateral margins of this half tube and is here somewhat thicker than usual; only a narrow proximal strip of the peduncle is uncalcified at this place. This thin-walled and wholly chitinous strip widens backwards to comprise a considerable part of the peduncle back. The border between the calcified and uncalcified parts appears as a distinct, oblique line.

The opposite side of the peduncle appears as excavated. From this side it can be seen that the strongly calcified margins of the semitubular peduncle part just described are the rims of the aperture field. Thus they do not form a semicircular border round the aperture field as in the avicularia of *Dendrobeania*, etc., but are prolonged downwards to the end of the peduncle without fusing. It follows from this that the aperture field is not restricted in the usual way to a small area of the avicularian front but extends downwards to the fixation area of the peduncle, getting by degrees narrower in accordance with the decreasing thickness of the peduncle. This uncalcified, delicate but tough membrane simultaneously becomes gradually more depressed below the level of its calcareous borders and reaches the attachment point of the avicularium on its mother zoid intimately close at the back wall of the peduncle, at a comparatively considerable distance inside the ends of its rim. Hence the excavated appearance of this surface. Thus a cross section of the lowest part of the peduncle is crescent-shaped.

After these introductory words on the structure of the avicularium we turn to its movements. They take place in the same way in the five forms examined

by the author. The description and figures refer to the conditions in *Bugula flabellata*, which possesses the largest avicularia of these forms.

The stage when an avicularium has bent forward to the extreme limit of this movement can be selected as the starting-point (fig. 3 A). The short peduncle is straight at this moment. The mandible is always closed. The adductor is so strongly contracted that large parts of the two abductors appear.

As the first sign of a movement beginning the huge adductor relaxes, now covering larger parts of the abductors than before (fig. 3 B). At the same time the first abductor contracts lowering the distal part of the frontal membrane, the mandible opens rather slowly, and the entire avicularium sways slowly backward. The membraneous ventro-proximal part of the peduncle gets a little shorter, forming a slight inward bend.

When the avicularium has passed between ⅓ and ¼ of the course of its full movement, the mandible stands partly open. Then the second abductor is seen to contract instantaneously, the large proximal part of the frontal membrane is suddenly lowered, the mandible opens completely with a jerk, and the avicularium swings swiftly backward along the remaining ⅔—¾ of its course (fig. 3 C). The inward bend of the membraneous peduncle part deepens rapidly until it is, during the last part of the movement, almost doubled, the opposite walls of the fold being tightly pressed against each other.

Then the characteristic swaying-to-and-fro-movement of the avicularium starts. The backward movement always reaches the extreme end of the route, the forward movement, however, only to the point described above as situated ⅓—¼ of the route from the extreme forward end of the latter (cf. MARCUS 1926 b, p. 90). The facts are significant that the mandible remains unmoved during this movement, standing fully open, and that the second abductor regularly contracts and relaxes simultaneously with the backward and forward phases of the movement, the proximal part of the frontal membrane regularly lowering and raising, whereas the first abductor and the adductor do not change, the former being contracted and the latter relaxed all the time.

When the mandible closes, which takes place rapidly with a snap, the whole avicularium instantaneously jerks forward to the extreme position taken as the starting-point of this description, as was observed by REY (1928). The adductor simultaneously contracts suddenly, both of the abductors relax, the whole of the frontal membrane raises, and the peduncle straightens.

CALVET's and MARCUS's explanations of this avicularian movement, questionable already from the fact that the special flexor muscles described by them do not seem to exist, do not fit in with the occurrences just described. According to CALVET's theory the peduncle ought to bend gently and evenly, according to MARCUS's theory its shape ought to be unchanged as was argued above. Further, the striking correlation of the movements of the whole avicularium and those of its mandible cannot be explained according to those theories.

VOL 31 195

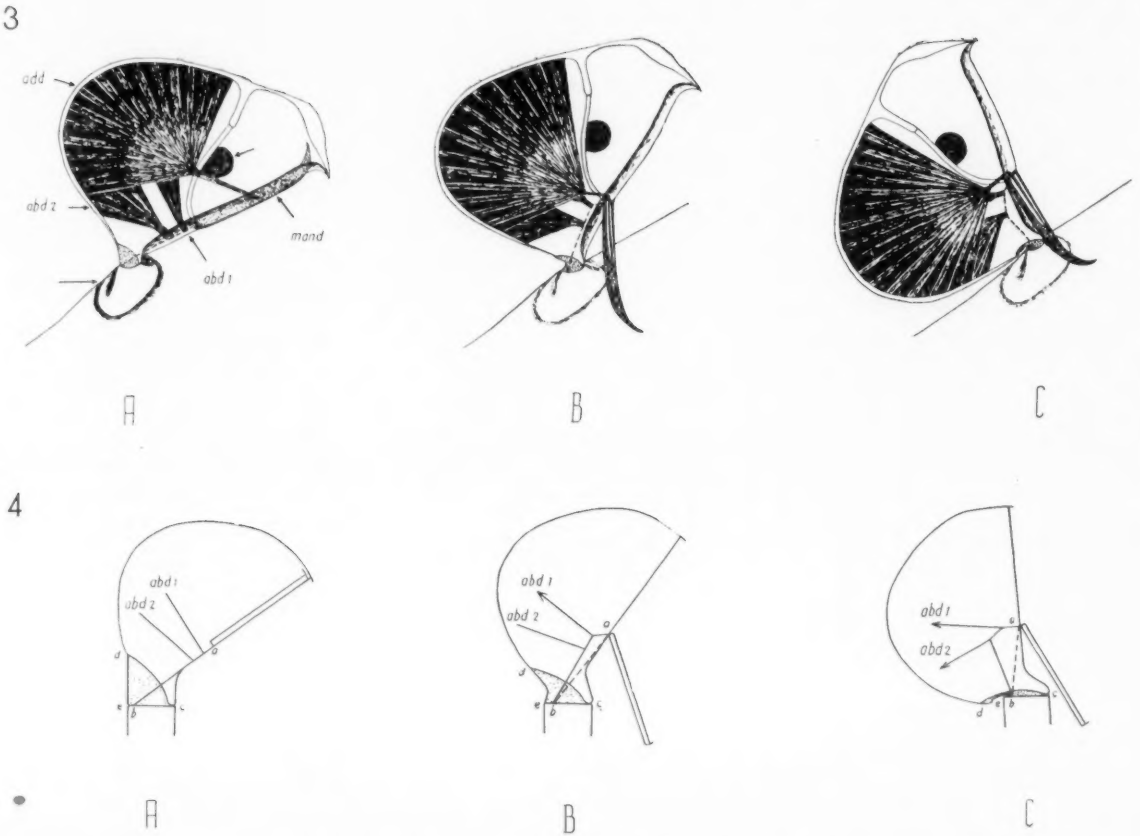

Fig. 3. *Bugula flabellata*. Different positions of avicularium in lateral view (cf. the text). Extension of frontal membrane indicated by broken line. Chitinous part of peduncle wall dotted. 180 ×. *abd 1*, *abd 2*, distal and proximal abductor; *add*, adductor; *mand*, mandible; *pol*, tactile body; *pro*, projection of mother zoid.—Fig. 4. Diagram constructed after fig. 3. For explanation see the text.

It is evident from the above description of the movements that they are caused by the abductor. This statement coincides with the correlation of avicularian movement and mandible movement. When the mandible is closed the adductor is, *a priori*, contracted and its antagonists, the abductor portions, relaxed. When it opens this means that the adductor relaxes, the abductors contract. When it stands open the adductor remains relaxed, the abductors can stay contracted or relax; actually it is so that the distal abductor portion remains contracted whereas the proximal one alternatingly contracts and relaxes. It follows from this that an avicularium with a closed mandible cannot move as the abductors are necessarily relaxed at this moment. When the mandible opens the avicularium must move too as the abductor contracts at this moment. The differentiation of the abductor into two portions is reflected by the details of the movements. The distal portion mainly effects the opening of the mandible, straining a small distal part of the frontal membrane; however, a slow and small backward movement of the avicularium takes place at the same time. When then the proximal portion contracts and the frontal membrane is even more depressed, the opening of the mandible becomes complete but the main effect is the complete and swift swinging backward of the avicularium. As the distal portion remains contracted as long as the mandible is open, we can now understand that the subsequent movements to and fro of the avicularium, which are caused by the alternating relaxation and contraction of the proximal portion, never exceeds, in the forward direction, the limit to which the avicularium was brought back in the first phase by the contraction of the distal portion alone.

The connection between the lowering of the frontal membrane by the abductor portions and the movement of the entire avicularium is thus clear. But how is this movement really effected?

The idea might seem sensible, from theoretical grounds, that it is effected by changes in the internal pressure of the avicularium caused by the alternating contraction and relaxation of the abductors thus lowering and raising the frontal membrane. However, this idea is definitely precluded by the following small experiment. With caution an opening can be made in the wall of the swollen part of the avicularium without injuring the muscles occupying it. The author has observed many avicularia perform their regular movements in spite of this free communication between their interior and the surroundings.

Thus it is obvious that the frontal membrane only serves to transfer the pulling power effected by the abductors—mainly the proximal one—to another part of the avicularium.

What really takes place is diagrammed in fig. 4 A—C constructed on fig. 3 A—C. The frontal membrane extends from *a* to *b*. When the distal abductor contracts the mandible is opened, and the point *a* is fixed. The point *b* is fixed from the beginning. The frontal membrane has been lowered a little, which means that the direct distance between *a* and *b* has been shortened. This means

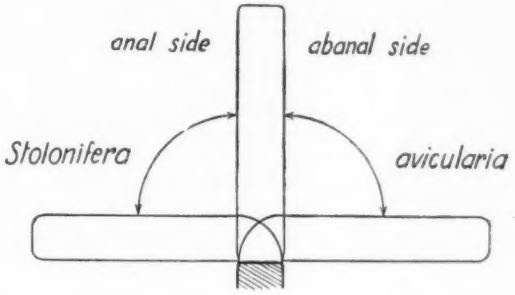

Fig. 5. Diagram to demonstrate flexion from erect position: towards abanal side in mobile avicularia, towards anal side in mobile autozoids of Stolonifera.

that the rigid unit consisting of almost the entire "head", on which the point *a* is situated, must come closer to the firm point—*c*—*c*—of the process of the mother zoid. This is not possible on the frontal (a b a n a l) side of the avicularium as the calcified margins of the aperture field reach that process at the point *c*, but can be done on the opposite (a n a l) side, where the peduncle wall is membraneous between *d* and *c*. This part is somewhat bent with the result that the head, turning round *c*, moves a little backward-downward towards *c*—*c*. When the proximal abductor then contracts the frontal membrane is lowered still further, the distance *a*—*b* is shortened even more, the "head" sways backward to its extreme position in that direction, and the membraneous part of the peduncle is sharply bent. The swaying to and fro of the avicularium then takes place between the stages C and B, the distance *a*—*b* alternately lengthening and shortening, and the extreme forward position of stage A is attained only when the frontal membrane is straight. It is hardly necessary to stress the fact that the mandible does not move during this latter action as the retractor is relaxed all the time. Several fine details of the structures connected with the mobility, especially the frontal membrane, can be suspected as remaining unobserved, which influence the action, though not such as have any importance for the main principles.

The elasticity of the uncalcified parts of the peduncle can certainly be assumed to be the power effecting the return of the avicularium to its original position.

If we take the erect position of the avicularium as the starting-point, it moves between this position and a position at right angles with the a b a n a l side (fig. 5). The abductors—in particular the proximal one—serve as e r e c t o r s. It is a peculiar fact that they insert in the abanal wall of the avicularium, whereas the erecting movement takes place in the opposite direction. This is made possible by transmission of the power of the erectors *via* the frontal membrane to a firm point on the mother zoid which is situated on the back (anal) side of the "hinge" formed by the stiff abanal peduncle pillars

resting on the mother zoid (figs. 3, 4). These features are essential for an understanding of the mechanism.

Though the details of this moving mechanism may seem complicated at the first glance, its principle is obviously quite simple. There are no fundamental differences of structure between a movable avicularium and an immovable one. Already the type of immovable avicularium found in, for instance, *Dendrobeania*, possesses the main qualifications required by the movable type, a free position, a narrow peduncle, and an abductor divided into a distal and a proximal part. Some small alterations in the structure of the peduncle wall, the extension of the frontal membrane to the fixation area of the peduncle and, to be more accurate, to a point there situated b e h i n d the margins of the aperture field, and the definite separation of the insertion points of the two abductor portions, are the small changes rendering the movement possible. The origin of movable avicularia from immovable ones thus affords no difficulties for the imagination.

VOL. 31 195

3. AUTOZOIDS OF CERTAIN STOLONIFERA.

A mobility of entire autozoids has been observed or assumed for several Stolonifera. They are as follows.

Fam. M i m o s e l l i d a e:

Mimosella gracilis Hincks: HINCKS 1851, pp. 359—360, and 1880, pp. 555 ff. Other members of *Mimosella:* JOLIET 1877, p. 293, and 1888, p. 106 (cf. HARMER 1915, p. 81); WATERS 1914, p. 852; HARMER op. cit., pp. 79 ff.; MARCUS 1938a, pp. 243—244, and 1938b, p. 58; SILÉN 1942, p. 31.

Fam. V a l k e r i i d a e:

Valkeria tremula Hincks: HINCKS 1862, p. 472, and 1880, p. 554; this species does not seem to have been recorded or studied by any other author and its affinities are not clear.

Farrella repens (Farre): MARCUS 1926b, p. 89.

"*Farrella atlantica* Busk": WATERS 1914, p. 853; according to HARMER (1915, p. 74) WATERS's specimens do not belong to this species but should possibly be referred to *Mimosella*.

Fam. T r i t i c e l l i d a e:

Triticella pedicellata Alder: ALDER 1857, p. 25.

T. koreni G. O. Sars (synonyms: *T. boecki* G. O. Sars and *Hippuraria egertoni* Busk): G. O. SARS 1873, p. 4; BUSK 1874, p. 30; HINCKS 1880, pp. 542, 549; MARCUS 1926a, p. 23, and 1940, p. 322.

In most of the few cases where a movement of the present type has been directly observed, this fact has been simply noted without further comments. Only HINCKS has given a description of it in *Mimosella gracilis*. In all the

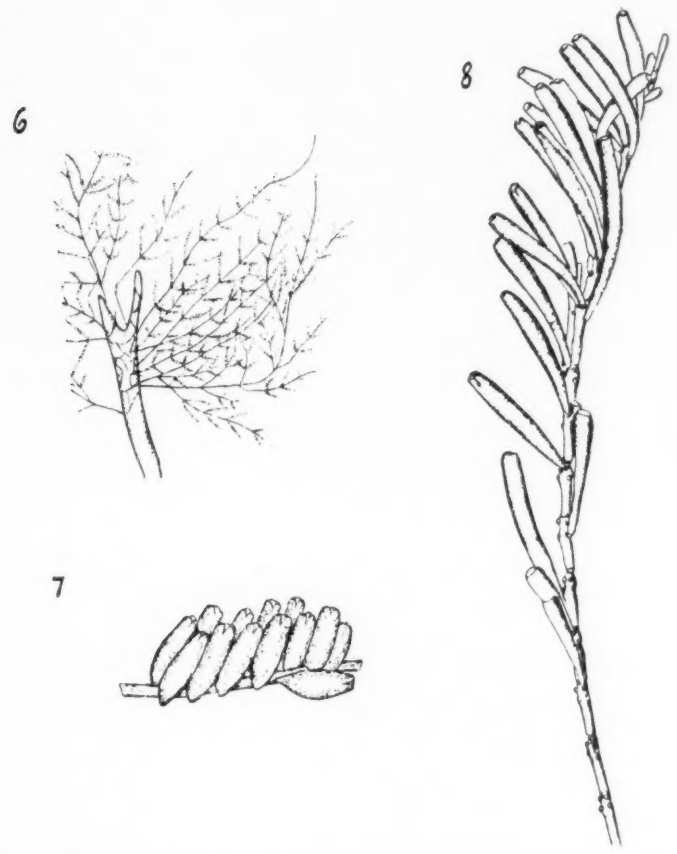

Fig. 6. *Mimosella gracilis*. Nat. size.—Fig. 7. The same. Part of branch showing one flexed and several erect zoids. 30 ×. This is the only picture of living mobile Stoloniferan autozoids in the literature.—Fig. 8. *M. bigeminata*. Distal part of zoarium. 33 ×.—Figs. 6—7 after HINCKS 1880, fig. 8 after HARMER 1915.

Stolonifera the stems, s t o l o n s, of the zoaria are composed of tubular kenozoids, as is well-known. The autozoids are fixed on the stolons by their proximal ends. The areas of attachment are perfectly circular. In the present species —and many others—a very small kenozoid is situated as an internode between the stolon and the autozoid. HINCKS observed that the autozoids can stand erect from the stolon, move down taking a position almost parallel to the latter, then swing back to their original position, and so forth. The movement takes place, according to him, between the hollow base of the autozoid and the internod. These movements of an entire zoid are coincident with the movements of its polypide, the latter protruding when the zoid swings to its erect position

and retracting when the zoid swings down again. Zoids without polypides are also able to move.

Later authors (JOLIET, WATERS, HARMER, MARCUS) have observed the special muscles—the "flexores zooecii" of HARMER—which effect the movement. These fibres are described as assembled into two bundles (in the only feebly moving *Farrella* with its few fibres such an arrangement does not seem to have been discerned by MARCUS), which in most forms are said to insert in the traverse wall separating the autozoid from the internode or stolon.

The opinion appears from the descriptions that the movements as a rule take place between zoid and internode or stolon as was stated by HINCKS. However, there are some exceptions. In *Mimosella tenuis* Harmer (HARMER 1915, p. 84, Pl. VII figs. 11—14) the proximal end of the zoid forms a narrow peduncle partly separated from the distal main part of the zoid by a joint in the body wall. At this joint, thus well within the zoid and far from its proximal end, the "flexor" muscles are inserted. In the *Triticella* species the proximal ends of the zoids form very long, stiff peduncles. The movement is said to take place between the "main zoid" and the peduncle. Finally, MARCUS (1938b, Pl. XIV fig. 34 B) pictures the "flexor" muscles as wholly situated within the zoid in *Mimosella verticillata* (Heller) var. *firmata* Marcus (cf. however, MARCUS's [1938a, fig. 32 B] picture of the corresponding conditions in *M. verticillata*).

There is some uncertainty as to several points in these descriptions, which appear as important for a true understanding of the movement. What is the real nature of the "flexor" muscles? Are there really two different types of mechanisms effecting the movement in, on the one hand, most of the forms considered here and, on the other hand, in *Mimosella tenuis*, *Triticella* and possibly others? Which are, in fact, the real details of structure and function in all the forms? And what is the connection between the movement of the entire zoid and the movements of its polypide?

Unfortunately, the present author has not had the opportunity of examining any of these rare forms in their living state. *Mimosella gracilis* was sought for at Plymouth and Marseille, though in vain.—*Triticella koreni*, considered by several authors as possessing movable zoids and being, in fact, of a general structure making such a conclusion seem probable, was examined at the west coast of Sweden. Though a quite fresh material of it, where all of the ordinary functions were normal, was obtained and scrutinized on several different occasions, I was disappointed to find no indications of a movement of the zoids. Dr. HASTINGS at the British Museum and the late Dr. DONS, Trondheim, have kindly informed me that they have had the same experience with material from Scotland and Norway respectively. A positive indication that the species is really immobile is found in the fact that the present author has caused its polypides to retract on numerous occasions but the retraction was never accom-

VOL. 31 1950

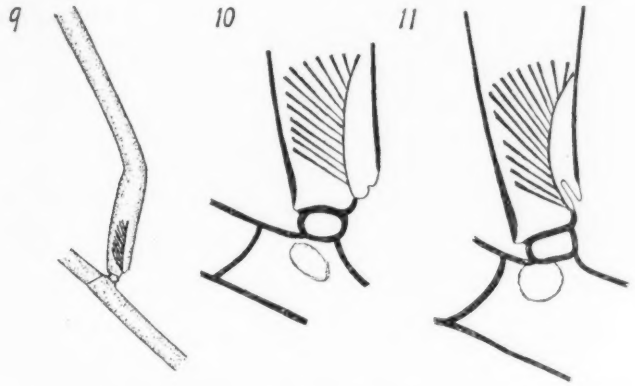

Fig. 9. *Mimosella bigeminata*. Zoid (artificially broken). 75 ×.—Figs. 10—11. The same. Proximal parts of partly and completely erected zoids to show erector muscle, extension of thinwalled and thickwalled areas, and connection with stolo. 300 ×.

panied by a movement of the whole zoid (cf. below). Possibly the old statements are based on specimens where the zoids were about to degenerate. On such occasions the "main zoid" above the peduncle degenerates as a whole in this species, being more and more loosely connected with the peduncle and swaying hither and thither with the water movements until it is, finally, completely detached; a new zoid is then regenerated from the top end of the peduncle. In living or preserved material of the species the author has failed to perceive the supposed flexor muscles pictured by MARCUS.

However, observations of importance in this connection were made on preserved material of *Mimosella verticillata* and *M. bocki* Silén (SILÉN 1942, pp. 30—31) and on HARMER's slides of *M. bigeminata* Waters, *M. verticillata*, and *M. tenuis* (cf. HARMER 1915, pp. 78 ff.) The material of *M. bigeminata* proved to be particularly instructive, and the following account is mainly based on it.

We have first to define the position of the zoid in relation to the stolon and thus also in relation to its own movements. The anus is situated on the side of the zoid turned to the growing tip of the stolon. This side is the a n a l or d o r s a l side. It is the side turned to the stolon when the zoid swings down towards the latter. The two rows of parietal muscles are situated in the opposite, a b a n a l or v e n t r a l, side; this is the case in the other Stolonifera examined by the present writer as well, and the Stolonifera thus agree, fundamentally, with the remaining Gymnolaemata in this respect.

Though the body wall of *M. bigeminata* has, generally, the thin and membraneous appearance characteristic of the Stolonifera, a close scrutiny reveals that in the proximal part of the zoid its structure is very different in different areas (figs. 9—11). Round its area of attachment to the small internode connecting

it with the stolon, it is thick and stiff within a strip, very narrow on the anal side but getting gradually a little broader towards the opposite side. Thus the wall of the lowest zoid end forms a stiff, shallow, eccentric cup firmly attached to the similarly firm underlying parts of the zoarium. Distally to this cup the body wall abruptly decreases in thickness and is very thin within a circular area forming a narrow strip on the anal side of the zoid but widening considerably towards the opposite side, where it comprises the whole of the projecting swollen part appearing in a zoid swung forward parallel to the stolon. The comparatively very thick wall of the remaining large distal part of the zoid contrasts sharply with the thin-walled area. The fact that thin-walled "islands" also occur within this large tubular part of the zoid—for example along a considerable part of its abanal wall functioning as an aperture field—does not affect the conclusion that it appears as a stiff unit against the thin-walled strip proximal to it.—In all the zoids holding an erect position the thin-walled proximal abanal bulge has disappeared and the walls form a deep inward fold in this place.

Each of the two fan-shaped "flexor" muscles is composed of a large number of fibres emanating from the interior surface of the proximal part of the stiff distal zoid tube except for the aperture field. The fibres attach, in each side of the zoid, to a tendon inserted into the abanally directed part of the distal margin of the proximal firm cup, thus at a small but distinct distance from the traverse wall separating zoid and internode.

The situation and mode of insertion of the "flexors" make it evident that they are serial homologues of the parietal muscles as was already recognized by JOLIET (1877, p. 293).

HINCKS's description of the movements performed by the entire zoid correspond so well with this analysis of the zoid structure that there cannot be much doubt that the movements take place in the following way (fig. 12). Nevertheless, it is of course highly desirable that these results should be controled on living material when somebody is lucky enough to obtain it.

In the zoid taking the position parallel to the stolon, the polypide is retracted and the abanal part of the proximal thin-walled region forms a swelling, as has been stated above. Then the "normal" parietal muscles contract lowering the frontal membrane and forcing the polypide to protrude. Simultaneously the special parietal muscles called flexors contract. As they emanate from the distal stiff portion of the zoid and insert into the proximal stiff portion, the effect of their work is that the two portions are brought closer together, which means in this case, as the proximal portion is firm, that the distal portion is drawn towards the latter. This is possible owing to the existence of the thin-walled region connecting them, the walls of which can be folded. As this region is narrow in the anal side it allows only a small—or possibly hardly any—movement in the proximal direction in this side before the distal stiff zoid

VOL.
31
1950

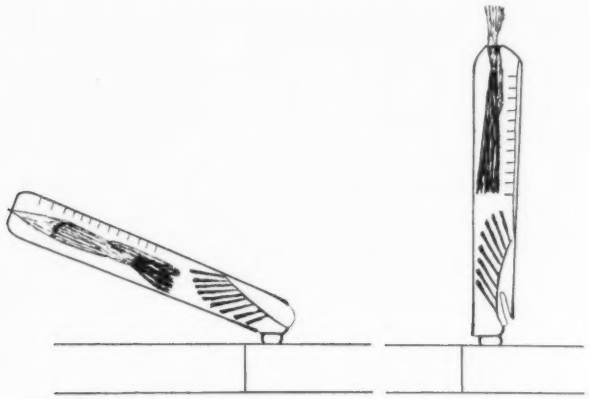

Fig. 12. Diagrams of Stoloniferan autozoid in flexed (left) and erect (right) position. Normal parietal muscles indicated in right sides of both zoids, in right zoid drawn as having lowered membraneous part of wall. For explanation see the text.

portion is brought in contact with the proximal portion. Owing to the considerable height of the region in the opposite, abanal side the distal portion is here moved an incomparably longer distance towards the proximal portion before it is stopped, the thin wall between them being compressed to form a deep fold. The result is obviously that the whole of the distal portion—comprising so large a part of the zoid that it seems to the naked eye to represent, in *M. bigeminata* and others, the whole of the zoid—is turned over to the abanal side. When the zoid is then excited, the retractor muscles contract retracting the polypide. Simultaneously the parietal muscles, including the "flexors", relax and the zoid swings back to its original position. This movement can be assumed, in all probability, to be effected by an elasticity of the thin-walled region making it straighten and resume its original shape when the pressure effected by the "flexors" ceases. Not any muscles antagonistic to the "flexors" have ever been observed and their presence is not probable for theoretical reasons either.

If we take the erect position of the zoid as the starting-point, the zoid thus moves between this position and a position at right angles with the a n a l side.

As the structure, in the respects now discussed, seems to be identical in all the forms examined and as the mode of moving exhibited by the movable Stolonifera agrees in general features, it can be assumed that this picture of the mechanism is valid for all the Stolonifera possessing this peculiarity.

It is seen that actually the movement does not take place between the zoid and the underlying internode but wholly within the zoid itself in *M. bigeminata* and, presumably, other forms of this type. Thus the peculiarity that the articulation takes place between the main part of the zoid and a narrow peduncle-

like proximal portion of the latter in *M. tenuis* and the like is no longer a peculiarity. The difference between the two types is only a difference of degree, being a difference in length of the zoid part situated proximally to the articulation.

Further, it is evident by now that the term "flexor muscles" is not fortunate. These muscles do not move the zoid downward towards the stolon but, on the contrary, make it move to the erect position. They should be called "erector muscles".

The simultaneity of the protrusion of the polypide and of the erection of the zoid and the reverse is obviously owing to the fact that the parietal muscles, including those specialized as erectors, effect both of the movements.

As is evident from the above, the movable form of autozoid in the Stolonifera can be quite easily derived from the typical autozoid of the group. The fundamental point is, simply, that a joint has appeared between the emanation points and insertion points of the most proximal pair of parietal muscles. These muscles have grown much stronger than the other parietal muscles, and certain parts of the wall have been reinforced thus facilitating and directing the movement.

VOL.
31
1950

We ought not to leave the present matter without having considered the interesting structure of the kenozoids composing the stolon in *M. bigeminata* to which HARMER (1915, p. 80) has drawn attention (fig. 13). The kenozoids are shaped like tubes. In the distal part of the stolon one or two pairs of autozoids or bases of detached autozoids are attached to the distal part of each kenozoid whereas the proximal kenozoids are devoid of autozoids. Between the zoids of the proximal pair (or proximally to the zoids when there is only a single pair) the kenozoid wall is membraneous within an oblong area contrasting with the remaining comparatively thick and stiff wall. A bundle of muscle fibres emanating from the opposite wall inserts into the membrane.

HARMER's interpretation of the phenomenon is: "It seems clear that the contraction of these fibres must increase the turgidity of the segment, and probably makes it sufficiently rigid to withstand the strain caused by the contraction of the muscles which move the zooecia."

This opinion seems sensible enough. It is true that the erector muscles of the autozoids do not insert into their proximal, traverse walls, as HARMER imagined, but the movement caused by them takes place wholly within the autozoid. Nevertheless, it can be imagined that some strain is effected on the kenozoid. The possibly significant fact can be added that membraneous areas of the same structure and corresponding position are present in the proximal kenozoids devoid of autozoids, but that no muscles are formed there.

However, such vertical muscles in the kenozoids of the stolon have been reported by some other authors (EHLERS 1876, pp. 17—18; JOLIET 1877, p.

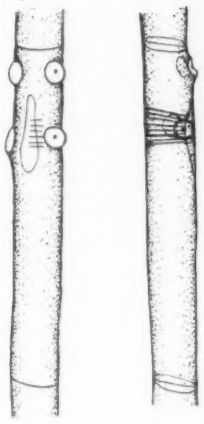

Fig. 13. *Mimosella bigeminata.* Kenozoid with four autozoid marks, frontal membraneous area, and muscles inserted in the latter, in oblique frontal (left) and lateral (right) view. Picture to the left after HARMER 1915. 160 ×.

293; WATERS 1910, pp. 239 and 250; MARCUS 1938a, p. 243 and fig. 32, 1938b, Pl. XIV fig. 34 B, and 1940, fig. 163 B and C) not only in other Stolonifera with movable zoids *(Mimosella verticillata, M. gracilis, Farrella repens)* but also in such forms where no movements of the entire zoids have been observed *(Hypophorella expansa, Valkeria uva, Bowerbankia imbricata)*. The present writer has examined the two last species and can confirm that their zoids do not move; on the other hand he has not succeeded in finding any muscles in their kenozoids. If they are really present, the problem of their function is puzzling indeed, and HARMER's theory is greatly affected if we do not make the assumption, which is rather daring in the present imperfect state of our knowledge, that these muscles have different functions in Stolonifera with movable zoids and those with immovable ones.

Here it is of some interest to note that MARCUS (1925a, 1925b) has concluded that the kenozoids forming stems in *Caulibugula mortenseni* (Marcus) are movable upon each other to judge from the otherwise inexplicable presence of parietal muscles in them and from the structure of their ends. This form belongs to quite a different part of the Gymnolaemata, the Bicellariellidae. Nevertheless, the similitudes in common for this form and the Stolonifera represented by the presence of stems composed of kenozoids and the presence of parietal muscles in them, could be imagined to indicate that this idea may be applied to the Stolonifera as well. The complicated structure of the ends of the kenozoids in *Mimosella bigeminata* at least could possibly be claimed as pointing in the same direction. However, in such forms as *M. verticillata, Hypophorella* and *Bowerbankia imbricata* the stolon adheres firmly to the substratum, which seems to exclude its mobility. All the same, as long as such movements have not been directly observed, this question must be handled with great caution.

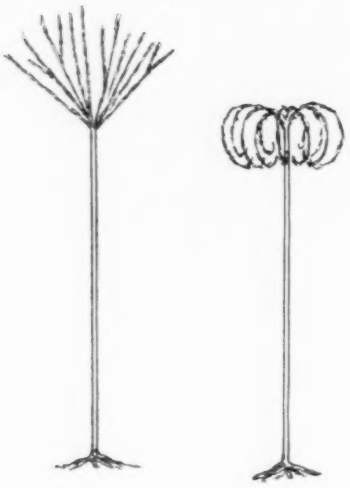

Fig. 14. *Kinetoskias smitti*. Diagrammatic pictures of zoarium with straight and with curved branches. After KOREN & DANIELSSEN's description and pictures.

VOL. 31 1950

On the whole it can be stated that the entire problem of the kenozoids' muscles is obscure at present and that much more facts are needed before a solution can be offered with any claim to reliability.

4. AUTOZOIDS OF KINETOSKIAS.

This genus consists of five species occurring mainly in deep water and obtained on rare occasions only. The sources of information on its structure and biology are as follows:

K. arborescens Kor. & Dan.: DANIELSSEN 1868; SMITT 1868, pp. 353—358; KOREN & DANIELSSEN 1877, pp. 107—109.

K. smitti Kor. & Dan.: DANIELSSEN 1868; KOREN & DANIELSSEN 1877, pp. 104—107; NORMAN 1893 and 1894.

K. cyathus (Wyv. Thomson) and *K. pocillum* Busk: BUSK 1881 and 1884, pp. 43—45.

K. elongata Harmer: HARMER 1926, pp. 468—470.

The present writer has examined a well-preserved material of, in particular, *K. smitti* but also of *K. arborescens*, which forms are especially considered in the following treatise. KOREN and DANIELSSEN are the only authors who have published observations on living material. The present author has tried to obtain specimens of *K. smitti* in the Trondheim Fiord, from where it has been recorded on comparatively numerous occasions, but in vain.

Generally expressed, the zoarium is composed of an erect peduncle fixed by its lower end to the substratum, and a bush of numerous, narrow, biserial

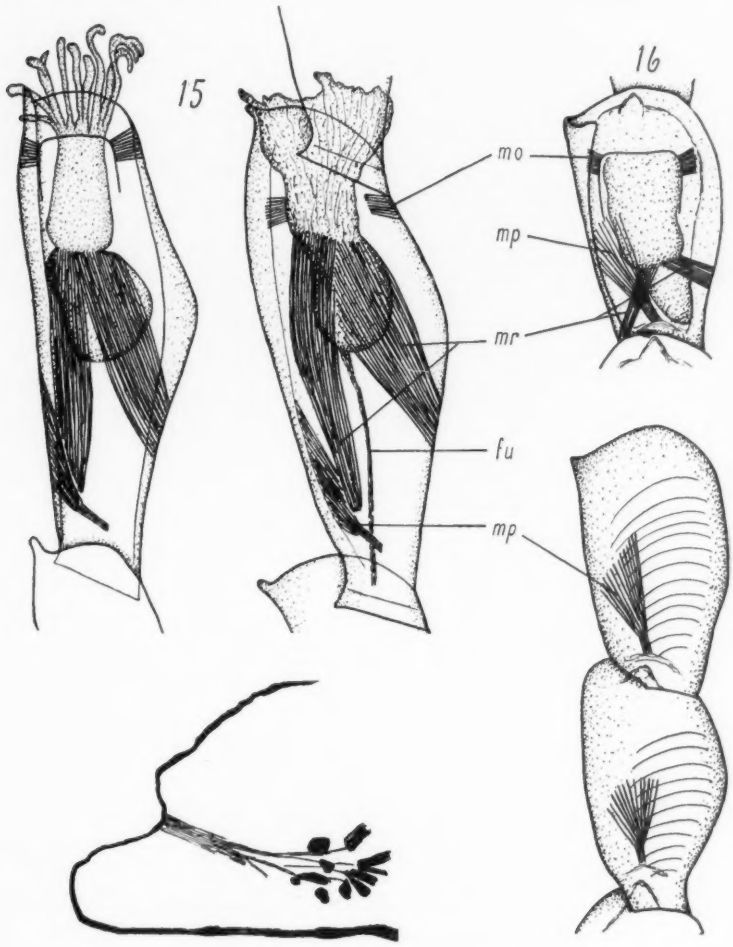

Fig. 15. *Kinetoskias smitti*. Zoids in frontal view (zoid from right row of bilinear zoarium) and in basal view (zoid from left row). 75 ×.—Fig. 16. *K. arborescens*. Zoids (from right and left rows respectively) in frontal and basal view. 75 ×.—Fig. 17. The same. Proximal part of zoid in oblique longitudinal section showing distal end of parietal muscle (erector) with tendon. 225 ×.—*fu*, funiculus; *mo*, occlusor; *mp*, parietal muscle (erector); *mr*, retractor.

branches emanating from its top end (fig. 14). The backs of the zoids are turned inward towards the longitudinal axis of the zoarium, their fronts outward.

According to KOREN & DANIELSSEN the movements take place in the following way. The branches of the zoarium alternately straighten, the layer of branches representing the surface of a more or less flat cone situated with

the top downward on the peduncle end, and curve outward. When the latter movement "is complete, all the branches are nearly rolled together, whereby the umbrella of the polyzoarium assumes a globular form".

The zoid is oblong, narrow at its proximal end and gradually broader towards its rounded distal end (fig. 15, 16). The whole of its front is occupied by the aperture field; here the body wall is uncalcified and membraneous. Its lateral and basal walls are rounded and pass evenly into each other. They are provided with a distinct but comparatively thin, continuous calcareous layer ending in a strong rim round the aperture field. In *K. arborescens* the basal wall is traversed by raised, curved striae.

Already KOREN & DANIELSSEN stated, in *K. smitti* and *K. arborescens*, the presence of a special, strong muscle in each zoid (later called m. flexor zooecii by HARMER). According to them the muscle extends in these species from a hollow process of the distal wall of the preceding zoid, where it is fixed by a narrow tendon, to the frontal membrane, where it is fixed within a wide area reaching far into the distal half of the zoid. According to BUSK the muscle has quite a different extension in *K. cyathus* and *K. pocillum:* "— — — arising from the front of the base of the zooecium (it) passes obliquely backwards and upwards, expanding in a fan-shaped manner, to be inserted into its hinder wall to the height of about one third or one fourth of the zooecium." The hollow process supposed by KOREN & DANIELSSEN to serve for the attachment of the muscle "is clearly nothing of the kind". HARMER described in *K. elongata* how the proximal end of the zoid is surrounded by a calcareous rim laterally provided with "a strong, vertical tooth, which serves for the origin of the *flexor zooecii*, which is inserted into the basal and lateral walls of the proximal half of the zooecium."—As to other muscles we only find in KOREN & DANIELSSEN's work some remarks (referring to *K. smitti*) as follows: "The retractor — — — is attached at the bottom of the zooecium, — — —. On each side of the tentacular sheath, there is a rather long retractor-muscle, which goes down and attaches itself on the interior surface of the zooecium some distance above the bottom (parieto-vaginal muscles). Besides these muscles, there is also a pair of parietal muscles."

An examination of *K. smitti* and *K. arborescens* has convinced the present author that in fact the "flexor" muscle of these species coincides with the description given by BUSK for other species (figs. 15, 16). From a fan-shaped origin at the lateral and basal walls of the proximal half of the zoid the muscle extends obliquely downwards and is fixed by means of a dense bundle of epithelial threads (the tendon of KOREN & DANIELSSEN) at the proximal part of the frontal membrane (fig. 17). The muscle has obviously no connection with the distal process of the preceding zoid (such a structure is not always present but often appears particularly in zoids of medium age or older). — KOREN & DANIELSSEN's statement of the wide extension of the origo of the

VOL. 31 1950

"flexor" into the distal half of the zoid is certainly based on a confusion with another muscle, *i.e.* part of the retractor (what is called the retractor by them is obviously the funiculus).—As to the assumption that in *K. elongata* the "flexor" would insert into a firm proximal projection, the present writer feels inclined to look upon it as due to some error considering the coincidence between the remaining species in this respect and the far from easy work of discerning the anatomical details of these animals if not sectioned. Possibly there has been a confusion with part of the retractor in this case as well.

The present author has not been able to find any parietal muscles of the usual type in the present species. The "pair of parietal muscles" and the "parieto-vaginal muscles" of KOREN & DANIELSSEN certainly represent nothing else but the mm. occlusores operculi and mm. parieto-diaphragmatici respectively.

The "flexor" muscle evidently belongs to the parietal muscle system as seen by its origin from the lateral and basal walls of the zoid and its insertion into the frontal wall, *i.e.* by its extension from one part to another of the zoid wall. The presence of the "flexor" coincides with the probable absence of "normal" parietal muscles. We can conclude that all the small parietal muscles appearing in most Gymnolaemata have here united into a single one. (We find similar unions of the parietal muscles into one or two large muscles in the abductors of the avicularia.)

It has been generally agreed that the movement is effected in the simple way that the "flexor" contracts and bends the basal wall of the zoid in the frontal direction. As no muscles antagonistic to the "flexor" are present, it has been assumed that the straightening of the zoid is due to the elasticity of its walls. —The zoids move, fundamentally, independently of each other. However, large parts of or entire branches can move simultaneously or almost simultaneously. KOREN & DANIELSSEN explain this phenomenon thus: "— — — as the cause which produces contraction in a muscle, most frequently acts simultaneously on many, the consequence is, that a great part of the branch, or even the whole of it, is put in motion. — — — the branch bends bit by bit, accordingly as more and more muscles are set in motion." To this can be added that the movement can be imagined, theoretically, to be influenced by an excitation which the change of position of one moving zoid may effect on the proximal zoid to which it is attached. All the same, the movement of a branch represents the sum of the separate movements of the single zoids.

The above picture of the moving mechanism of the single zoid cannot be accepted for several obvious reasons. As the "flexor" is not, in fact, inserted into a firm structure but into the frontal membrane, it follows that its contraction cannot effect that the comparatively stiff basal wall is brought nearer to the former but effects, on the contrary, a lowering of the frontal membrane. Even if this were not the case, it could not bend the basal wall as the flexure

mainly takes place in the distal part of the latter (cf. below) whereas the muscle emanates from its proximal part only. Finally, as the frontal membrane is lowered by its contraction, the volume of the cystide must diminish. Consequently, its contraction must be simultaneous with the protrusion of the polypide (as no other parietal muscles seem to be present, it directly effects, in all probability, the latter movement). But the polypide protrudes when the zoid is straight (cf., *i.a.*, KOREN & DANIELSSEN 1877, Pl. 3 fig. 13), which must be the case also for the reason that the apertures of the zoids are turned inwards in the rolled-up branches and their polypides if protruded in these circumstances would obtain a most unfavourable, not to say impossible, position for their nutritive activity.

We must obviously look upon the work of the "flexor" from a quite different point of view.

What is, in fact, the shape of the zoid walls when "at rest", *i.e.* when unaffected by the power effecting the movement now in question? This problem has not been considered previously, but is obviously fundamental for the understanding of the mode of work of that power.

In order to settle this question the frontal membrane and the "flexors" were removed from several zoids. In this way all strains possibly effected on the remaining firm lateral and basal walls were eliminated. All zoids treated in this way take a curved position.

Consequently, the effect of the power causing the movement must be a straightening one contrary to what has been assumed.

Undamaged, curved zoarial branches have been placed into decalcinating fluids containing nitric or acetic acid. The comparatively thin calcareous layer of the zoids is dissolved in a short time in a 5 per cent solution and only their chitinous layer is left. Simultaneously the branch uncurves with such a rapidity that the movement is easily perceptible even to the naked eye. It finally passes beyond the straight position and is curved backwards. A close examination reveals that the inflection takes place in the distal half of the zoid; more proximally it is imperceptible. It is of no importance if the branch is removed from alcohol or water into the decalcinating fluids. Thus the increase or decrease of the internal pressure has no influence upon this phenomenon (cf. below). Moreover, the result was the same in zoids deprived of their frontal membranes and "flexors".

It is evident from this experiment that whilst the calcareous layer is curved forwards (in the frontal direction) if undisturbed by external powers, the chitinous layer of the distal half of the basal body wall has a structure making it curve backwards (in the basal direction) if isolated from other factors. As the calcareous and the chitinous layers are intimately united into one wall but endeavour to curve in opposite directions, this results in a strain within the wall.

VOL.
31
1950

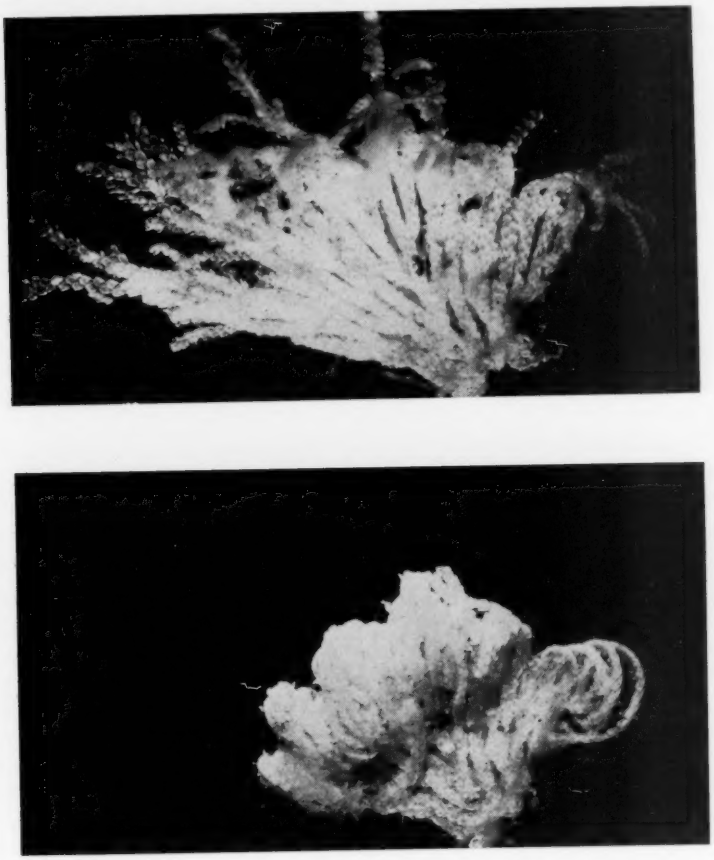

Fig. 18. *Kinetoskias arborescens*. One and the same (incomplete) zoarium placed in alcohol (upper), and just placed in water (lower). 3.5 ×.

The power of the calcareous layer is stronger and makes the wall (and the whole zoid) curve forwards when unaffected by external powers. However, the difference in strength between the two opposite powers is small as demonstrated by the fact that in the above experiment the zoid begins to curve backwards almost simultaneously with the beginning dissolution of the calcareous layer. — After the decalcination (as well as in sections) it is seen that the transverse striae of the basal wall of *K. arborescens* represent thickenings of the chitinous layer.

Thus there is a lability in the frontally curved position normally held by the basal wall indicating that even a slight external influence upon the wall, working in the same direction as the strain of the chitinous layer, is to be expected to make the wall (and the zoid) curve backwards.

It must be concluded from the above that the so-called flexor muscle has not the function ascribed to it but is, in fact, a m. e r e c t o r z o e c i i.

Then the problem is: in what way does this muscle effect the straightening of the zoid?

It is much stronger than parietal muscles of the usual type even if their united strength is considered. It might then be assumed that the frontal membrane lowered by it effects such a strong pressure on the body fluid that it not only forces the polypide to protrude, which is its normal function, but that the pressure also continues after the conclusion of that phenomenon. It does not seem impossible that exactly this continued pressure could effect the straightening of the zoid. When the frontal membrane is fully drawn in and thus fixed, the distal part of the basal wall remains as the only wall that can give way to the pressure.

It would be of interest then to settle what influence a pressure effected on the interior surfaces of the zoid walls really has on the shape of the zoids. For that purpose zoaria have been removed from alcohol (or a solution of salt) into fresh water and the reverse. In the former case the osmotic pressure increases temporarily in the zoids, in the latter case it decreases.

VOL.
31
1950

All the zoaria examined by the present writer are preserved in alcohol and are wholly rolled up. When such a zoarium is removed into water, it instantly begins to unfold rapidly, a movement that does not stop before the branches are straight; this state is reached in one or two minutes (fig. 18). If the zoarium remains in water it then begins to curl up again very slowly. If a zoarium, lying in water and being in a completely straightened state, is removed into alcohol it curls up again in few minutes.—A salt solution has the same effect as alcohol.

The effect upon the single zoid if removed from alcohol into water is that the distal half of its basal wall straightens making the distal zoid half unroll. A secondary effect is, of course, that the emanation-point of the next zoid in the row is simultaneously moved backwards.

The same phenomena are met with in the very ends of the zoarial branches, if treated in the same way. Here the lateral chitinous tubes, so-called rootlets present along the proximal parts of the branches, are not yet developed. Thus the theoretical possibility is excluded that the rootlets have something to do with the movements described.

This experiment can be repeated an unlimited number of times with one and the same zoarium.

The movements caused in this way exactly correspond to those performed by the living zoarium.

The first time a zoarium is removed from alcohol into water, the greater part at least of the retracted polypides are evaginated simultaneously with the

straightening of the zoids. For obvious reasons the polypides cannot retract again in these dead zoaria.

If these movements, the straightening of the zoids and the protrusion of the polypides, can be easily effected by means of an increase of pressure in dead zoaria, where the fixed condition of the tissues can be supposed to afford a certain resistance, they certainly can be performed even more easily in the living ones according to the same principles.

The facts advanced indicate that the mechanism of the movement of entire zoids in *Kinetoskias* is as follows:

The frontal membrane is depressed in its proximal part by the contraction of the parietal muscles united into the erector. At first the pressure is compensated as long as the polypide moves outwards. Even the full protrusion of the polypide, however, can be assumed as being insufficient to compensate the pressure as indicated by the unusual strenght of the erector muscle. The proximal part of the frontal membrane is drawn in by the muscle and cannot expand. The curved position of the distal half of the basal wall is very unstable and the pressure forces it backwards.—The reversion of the zoid to its original state after the relaxation of the erector is owing to the elasticity of its basal wall working in the forward direction.—It is to be noted that the emanation areas of the retractor portions are situated in the immobile proximal part of the basal wall (figs. 15, 16).

Busk (1884, p. 17), Robertson (1921, p. 39) and Harmer (1926, pp. 390—394) have treated the abyssal species *Euoplozoum cirratum* (Busk) belonging to the family Euoplozoidae closely related to the Bicellariellidae. Its erect zoarium is composed of bilinear branches. Robertson and Harmer have assumed that the zoarium performs the same curving and straightening movements as *Kinetoskias*, a supposition that is based on the likeness in the structure of the zoid particularly with regard to the arrangement of the muscles.

It is characteristic of the moving mechanism of *Kinetoskias* that the erector muscle does not work directly or via firm structures but by means of an increased pressure effected on and transmitted by the body fluid, and that the movement is not performed within a restricted joint contrasting with the remaining rigid part of the zoid but by means of a straightening and curving of the whole part that moves, which obviously is connected with the nature of the power working. This type of autozoid is easily derived from the general autozoid type of the Bicellariellidae by means of the simple changes that the parietal muscles have united into a single, very strong one of special position and that the distal large part of the basal wall has become unstable and flexible.

5. RELATION TO ORDINARY MUSCULAR ACTIONS OF AUTOZOIDS; STIMULI; CO-ORDINATION PROBLEM.

For a full understanding of the actions treated above, an analysis of the ordinary "active" muscular movements of the Gymnolaematous autozoid, which have reference to those actions, is necessary as a background.

They are: the protrusion of the polypide, indirectly caused by the contraction of the parietal muscles lowering the frontal membrane; the retraction of the polypide, directly caused by the contraction of the retractor muscle; the closure of the operculum, directly caused by the contraction of the occlusor muscles.

The effect of the parietal muscles and that of the retractor are antagonistic. The work of the occlusors is co-ordinated with that of the retractor.

The contraction of the retractor is, generally, caused by external, in natural conditions certainly mainly mechanical stimuli reaching the protruded polypide (in certain circumstances such as when an ovum is about to leave the body cavity internal stimuli have a similar effect, cf. SILÉN 1944). The contraction of the occlusors follows immediately on the retraction of the polypide. The contraction of the parietal muscles appears as caused by an excitation following the cessation of the stimulation that has effected the contraction of the retractor.

It is evident from the above that the normal, resting state of the zoid is represented by the expanded state. This point of view coincides with the nature of the protruding part of the polypide as the distal end of the animal. It must be stressed that this is a fundamental point of view as, from a more practical point of view, the term "resting" is somewhat inadequate in so far that the expanded state might seem the active state regarding the capture of food. The latter action, however, is in the Bryozoa—as is well-known—of a type not differing fundamentally from the activities of other parts of the feeding apparatus. Further, the cilia move incessantly in the retracted tentacle crown as well.

As is seen from the preceding chapters the movements of entire autozoids in *Kinetoskias* and the Stolonifera are effected by specialized parietal muscles, they certainly take place simultaneously with the movements of their polypides, and are in all probability caused by the same stimuli and cessations of stimulation which cause the polypide movements. No further argument is necessary to motivate a characterization of the movements of these entire zoids as representing no more than a special expression of the normal actions of the zoids.

This argumentation is also valid, to a certain extent, for the avicularia in general. As the polypide is rudimentary, its protrusion and retraction movements have been obliterated. However, parts of the muscle actions connected with these phenomena are retained.

The closure of the avicularian mandible by the adductor muscles obviously corresponds to the work of their homologues in the autozoid, the operculum and its occlusor, and, consequently, to part of the mechanism connected with the retraction of the polypide of the autozoid. The stimulus causing this effect is the same in both cases: a mechanical excitation of the distal part of the polypide, in the avicularium reduced to a "tactile body".—It is difficult to discern, in the autozoid, if the retraction of the polypide (=the contraction of the retractor) and the closure of the operculum (=the contraction of the occlusor) are effected by the same stimulus causing direct impulses to each of these muscles or if the latter effect is secondary to the former one (it could, for instance, be presumed as being caused by the ceasing of the stimulus represented by the protruded polypide touching the operculum). The fact that in the avicularium, where the former action is eliminated, the thus isolated closure action follows immediately on the excitation of the polypide may indicate that also in the autozoid the closure effect is brought about directly by the excitation of the polypide.

The parietal muscles (abductors) still effect the lowering of the frontal membrane in the avicularia. The secondary effect, however, is not the protrusion of a polypide, for obvious reasons, but the opening of the mandible. The homologue of the latter in the autozoid, the operculum, is opened by the protruding tentacle bundle (SILÉN 1938, p. 171), a mechanism that evidently cannot function in the avicularium. The stimulus causing this modified final effect is the same as that starting the protrusion of the polypide in the autozoid: the cessation of external mechanical stimulation. The condition at rest of the avicularium is the same as in the autozoid, as shown by the open position of the mandible when the avicularium remains undisturbed.

In the movable type of avicularium another secondary effect is added, the shortening of the distance from the proximal mandible end to the fixation point of the avicularium causing its movement.

After this analysis and comparison of autozoid and avicularium, the movements of the mandible and the movements to and fro of the entire avicularium correlated with the mandible movements appear as directly and simply corresponding to actions of the autozoid.

However, against this background appears surprisingly one type of muscle action in the avicularium: the regular swaying to and fro with open mandible between the points 2 and 3 of the figures 3 and 4. This movement is not correlated with the mandible movements more than in so far that, for natural reasons, it ceases when the mandible is closed. It is performed by the proximal group of parietal fibres fully separated in the movable avicularia.

It is peculiar in the way that it takes place during what is in all other zoids a period of rest and that the contraction and relaxation of the muscle takes

place alternately, incessantly and rather regularly (the rhythm is described by MARCUS 1926b, p. 90, and REY 1928) without any stimulus from the surroundings or impulses connected with actions of the other muscles being necessary for bringing it about.

This fundamental fact is not opposed by the interesting fact that it seems possible to influence the swaying movement by external means. REY (1928) has demonstrated that its rapidity can be accelerated or diminished by thermical and chemical means (his experiments with mechanical stimuli have reference only to the movement of the mandible). The present author has observed that in a colony strongly excited as a whole by having been shaken or lifted for a moment over the surface of the water, all avicularia perform strikingly rapid movements for a minute or two. Unfortunately, he has not succeeded in revealing what is the exact nature of the stimulation. It does not seem to be a stimulation of the tactile body as this causes the closure of the mandible which stops the movement of the whole avicularium, nor with impulses emanating from the adjacent zoids (cf. further below).

A similar phenomenon can be found in certain autozoids under special conditions. HINCKS (1851) and MARCUS (1926b) have reported that the zoids of *Mimosella* and *Farrella* respectively are able to move even when their polypides have degenerated. In normal zoids the contraction and relaxation of the usual parietal muscles are correlated with the relaxation and contraction of the retractor as argued above. The correlation between the movements of the polypide and of the entire zoids of the mobile type makes it seem probable that this is also true for the special parietal muscles serving as erectors. As is evident from the present example, where the normally probable source of impulses is absent, this correlation cannot be absolute in all circumstances. The fact is particularly worthy of notice that the nerve centre situated in the polypide in all Bryozoa must have degenerated with the latter. The details of the present action have not been studied by anybody.

To my knowledge an action of this type is only found in the Bryozoa in one more case, *i.e.* in the vibracularia of certain Scrupocellariidae. Here, however, it is represented by quite a different movement. In these heterozoids the operculum is transformed into the long, bristle-like seta. As has been described by several authors, the seta sweeps to and fro along the surface of the adjacent part of the colony, removing noxious matter (fig. 19). It is moved forwards by a strong adductor, the homologue of the occlusors of the autozoid, and backwards by an abductor, the homologue of the parietal muscles (CALVET 1900, p. 255). The polypide of the vibracularium has been described as transformed into a tactile body of the same type as in the avicularium.

MARCUS (1926b, pp. 56 ff.) has given an interesting description and careful analysis of the movement of the seta in *Scrupocellaria scruposa* (L.). According to him the impulse starting the movement is caused by the stimulation of the

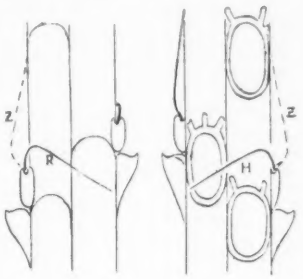

Fig. 19. *Scrupocellaria scruposa*. Part of zoarium in basal (left) and frontal (right) view, to show position of vibracularia and extreme basal position *(R)*, vertical position *(Z)* and extreme frontal position *(H)* of setae. After MARCUS 1926b.

frontal membrane of the autozoid on which the vibracularium is situated. The autozoid would consequently send impulses to the vibracularium. The movement of the seta would thus be caused by a mechanical stimulus as is the case with the avicularian mandible, which could seem quite natural as these organs and the respective muscles are homologues.

However, the present author, when examining *S. scruposa* and *S. reptans* (L.), has come to a result differing in one detail, which is of importance in the present connection. In my experience the setae sway to and fro even if the zoarium is not excited mechanically and all other special external stimuli can be judged as being eliminated. Mechanical stimuli only effect an acceleration of the incessant movement (in the present author's experience the stimuli must be effected on the vibracularium itself, cf. further below). Thus this action is apparently of the same type as the swaying-to-and-fro movement with open mandible of the avicularium. This fact was a surprise to the author, considering MARCUS's results as well as the different conditions in the closely related genus *Caberea*, which will be described below.—HINCKS (1880, p. LXXXI) seems to have come to the same result as he says that the movement of the seta "goes on uninterruptedly during the life-time of the colony". This statement refers to the "most highly specialized forms" of vibracularia "placed on the dorsal surface of the zooecium", which must mean the Scrupocellariidae and probably just *Scrupocellaria* as the same movement in *Caberea* is the subject of a special treatise later in the same work.

The behaviour in the present respect of the excessively developed vibracularia of *Caberea* (figs. 20, 21) is not clear from DARWIN's (1839, Ch. 9, May 19th, and 1859, Ch. 7) and HINCKS's (1880, p. LXXXII) descriptions. REY (1928, p. 16) found that normally the setae move here only after mechanical stimulation (in a 0,08 per cent solution of potassium chloride, however, they are said to beat regularly once in one or two minutes). This agrees with my own observations on *C. ellisi* (Flem.) from the Swedish west coast and *C. boryi* (Aud.) at Marseille.

Several other forms possess vibracularia but nothing seems to be known about their mode of work.

In two of the cases related—mobile avicularia, empty mobile autozoids of Stolonifera—the muscles acting without discernable influences from other actions or the surroundings are specialized parietal muscles. The case of the avicularia is especially peculiar as only the proximal part of these muscles (the second abductor) reacts in this way whereas their distal part (the first abductor) reacts in the usual way of parietal muscles, *i.e.* on the cessation of the stimulus having caused the occlusor (adductor) to contract. In the vibracularia of *Scrupocellaria* the antagonistic adductor (occlusor) and abductor (parietal muscles) both partake in the action.

As the structure of the nervous system is wholly unknown in the avicularia and vibracularia and fragmentarily known in regard to the muscle innervation of the autozoid (Gerwerzhagen 1913; Marcus 1926b, 1926c, 1934; Graupner 1930), the foundation is missing for giving a satisfactory explanation of the curious type of muscle action now discussed. Is it possible that the parietal muscles, or more exactly the erectors, of the Stolonifera in question have a double innervation, by nerves extending directly from the ganglion—which is absent precisely in the zoids with degenerated polypides—as well as by the parietal nerve net present at least in *Farrella* (Marcus 1926b) and said to be partly motoric at least in the Phylactolaemata? It can be inferred from the conditions in the autozoids that a ganglion is situated in the reduced polypide of the avicularia and vibracularia in general. The function of the latter as a tactile body can possibly be claimed to support such a supposition. However, precisely in *Scrupocellaria* the present author has failed to discover such a function. Can it be that a ganglion is absent there and the muscles are innervated by a diffuse nervous system? In the mobile avicularia the adductor and the distal abductor react instantaneously to a stimulation of the polypide and on the cessation of the excitation of the adductor respectively and can be assumed to be innervated directly from the ganglion. The proximal abductor, however, begins its work definitely later than the distal one, a comparatively considerable time after the relaxation of the adductor. Can it be that it is innervated in a different way, for instance by a diffuse nerve net? As a support for this proposition could possibly be taken the above related fact that the swaying movement of the avicularium can be accelerated by chemical, thermical, or mechanical means. At least the latter ones are not perceived by the polypide in this case as there the response to a mechanical stimulation is the contraction of the adductor. Is then any other possibility left than that these stimuli are perceived by sense cells diffusely spread in the body wall and connected with a parietal nerve net? If the latter really contains motor components as well, a direct transmission of the excitation to the proximal abductor

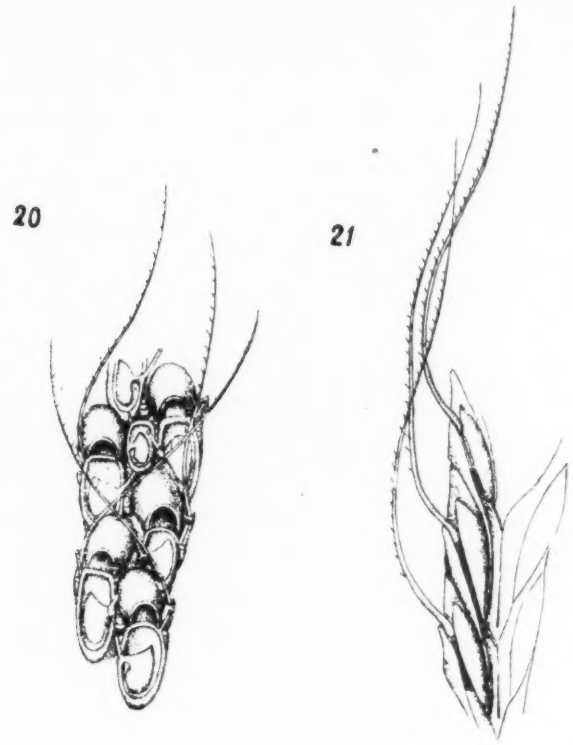

Fig. 20. *Cabrera boryi*. Part of zoarium in frontal view to show distal ends of vibracularia and setae in frontal position. 37 ×.—Fig. 21. *C. ellisi*. Part of zoarium in basal view with vibracularia and their setae. 25 ×.—After Hincks 1880.

can be imagined. If these considerations were true they should also have reference to the corresponding actions of the other zoid types discussed.

As is seen only guesses—or rather questions—can be advanced at present with respect to this problem. However, they may add somewhat to the analysis of the phenomenon now in question, pointing out as they do some accessory phenomena possibly of importance.

A special problem is the work of the parietal muscles described from certain kenozoids of the stolons of several Stolonifera (p. 364). In what way can they be stimulated to action? Generally, no definite organs are differentiated in the kenozoids, thus not even a polypide or polypide rudiment, the body part containing, in all Bryozoa, the ganglion and being the most important receptor of external stimuli. Is then a nervous centre absent in the kenozoids (cf. the Stoloniferan zoids where the polypides have degenerated)? Do they possess

other parts of a nervous system? We know nothing about this question. As the kenozoids in general do not perform any actions, this is hardly a problem of importance before we consider just the present exceptional type of kenozoids. And with respect to the latter comes the additional trouble that, in fact, no observations on their function in the living state have been made. To put it briefly, no f a c t s are available for the solution of the present problem. Could it be guessed that impulses are transmitted to the muscles of the kenozoids from the autozoids situated on it? This does not seem very probable as will be seen from the discussion of the co-ordination of different zoids following below. Or may the changes of position of those autozoids influence the kenozoid somehow? However, we have then to bear in mind that kenozoids with muscles have also been described in Stolonifera with—as far as is known—immobile autozoids.

We arrive, finally, at the question: are the actions of the several mobile zoids of a colony co-ordinated? Statements in that direction are met with in the literature. However, the kernel of this problem obviously lies deeper: is there a co-ordination of the actions in general of the members of a Gymnolaematous colony?

Against earlier opinions MARCUS (1926b, p. 54) demonstrated convincingly that, in the Gymnolaemata, a zoid does not respond to a mechanical stimulation of one of the adjacent zoids. This is wholly clear if one is careful that the stimulus does not affect more than one single zoid, which is not an altogether easy task in the throng of individuals in a colony. It is true that he found sensibility and a nerve-net in the cystid of *Farrella* but there were no connections with the neighbouring zoids. (The Phylactolaemata differ to a certain degree in this respect: an excitation of the cystid wall causes impulses to adjacent zoids, an excitation of a polypide does not; cf. MARCUS l.c. and 1926c, pp. 327 ff.)

However, HILLER (1939) briefly reported that he had succeeded in staining, in two species of *Electra*, nerve elements considered to represent connections between different zoids and has also found "that the polypides frequently react in groups to the stimulation of the frontal cystid wall, especially in the region of the operculum." To my knowledge this notice has not been followed by a more detailed treatise.

The results of the present author's experiments with autozoids of various Gymnolaematous forms agree clearly and completely with those of MARCUS: no transmission of excitations from zoid to zoid was observed.

In the behaviour of the mobile avicularia and the vibracularian setae of various forms DARWIN (1839, Ch. 9, May 19th) found reason to assume a transmission of impulses throughout the colony. HINCKS (1880, pp. LXXXIII

VOL. 31 195

and 58) remarks that in *Caberea* "the movements are synchronous" "obedient to a common impulse"; however, "the vibracula of *Scrupocellaria* act independently of one another." According to the same author's description (p. 558 and Pl. 74 figs. 2, 5) the movements of the autozoids of *Mimosella* are not co-ordinated, and KOREN & DANIELSSEN have demonstrated the same for *Kinetoskias*. To these statements should be added the observation of MARCUS already quoted that a mechanical stimulation of the frontal membrane of an autozoid in *Scrupocellaria* starts the movement of the seta of the vibracularium situated on that autozoid.

In all of the cases where a transmission of impulses from zoid to zoid has been claimed to take place, the experiments of the present author have entirely failed to confirm those propositions, a result that is quite natural against the background of the above related reactions of the autozoids of the Gymnolaemata in this respect.

The case of *Caberea* is a little more complicated than the other ones and deserves a few additional words. If a seta in the middle of a row of setae is mechanically excited in *C. ellisi* or *C. boryi*—the two species examined by the writer— one is at first astounded to see how, in fact, a large number of setae instantly partake in the rapid and strong sweeping movement. This is seemingly a clear confirmation of DARWIN's and HINCKS's observations. Often the movement spreads to other branches and the whole of the zoarium appears to be in a lively state of action.

However, the following facts are to be observed. The adequate stimulus of the *Caberea* vibracularium is, according to the present experiments, a touch on its seta. No other stimuli had any effect. The setae are wholly chitinous structures and have themselves, of course, no sensibility. The touch can be assumed, in all probability, to make the seta effect a pressure on the reduced polypide (tactile body) situated close to its base. This body seems to have such a sheltered position that it is not easily reached by direct mechanical stimuli. Even a slight touch on the seta is sufficient to bring about a movement (if the seta has not recently moved; a resting pause is necessary between each action, the length of which increases the more often the vibracularium has been excited within a certain space of time). The vibracularia are densely placed, and their setae are very long (figs. 20, 21). Consequently, when one seta moves it always touches at least the seta distal to it in the same row of vibracularia, often other setae as well. In fact, the seemingly simultaneous movement of a group of setae is not wholly synchronous in this case but slightly though distinctly metachronous.

In these circumstances the assumption is close at hand that the almost simultaneous action of a group of setae is effected in the way that the touch of one moving seta on the seta distal to it stimulates the latter to action, this in its turn stimulates the seta distal to it, and so forth. The following experiments

prove that this is really the case. If in a row where the setae do not reach other setae than those situated distally to them, one seta in the middle of the row is stimulated, only that seta and the setae distal to it move. If the most distal seta of the row is stimulated, no other setae move. If in a row all setae have been cut except one in, for instance, its proximal part and only stumps of them are left sufficient to show their actions, if in this row the remaining single seta is brought to action the stumps of the other setae do not move.

If a *Cabercа* colony is placed flat in a dish with all the setae resting against its bottom and the dish is then shaken, a synchronous movement of all setae follows. In this and similar cases the synchronism is evidently due to a simultaneous stimulation of all vibracularia directly.

Thus even in the supposed beautiful example of co-ordinated zoid actions of *Cabercа* no transmission of impulses from one zoid to another by the nervous system can be perceived but we have to do with external mechanical stimuli effected by the vibracularia on other vibracularia or with a simultaneous stimulation of several vibracularia. This case is fundamentally the same as when in the densely crowded autozoids of a Gymnolaematous colony, one protruded polypide happens to touch another one with the result that they suddenly both retract, an action that often spreads over considerable parts of a colony, or when a large number of extended polypides are reached by the same stimulus and retract simultaneously.

Also against this background MARCUS's assumption, that the muscles moving the entire avicularium in *Bugula* are situated in its mother zoid, seems questionable. If so, how could there be such a perfect co-ordination of this movement and the other actions of the avicularium? And, on the other hand, how is it possible that all sorts of stimulations of an autozoid have no effect on the movements of the avicularium?

Finally, it may be noted that, according to the present author's experiments with *Pedicellina* (Entoprocta), only the directly stimulated animal reacts. If the stimulation is carried further in the colony this is due to the bumps which that animal gives its neighbours during its convulsive movements, these neighbours likewise to their neighbours, and so forth.

6. SUMMARY.

The present author has come to the following conclusions regarding the actions of the different mobile zoid types of the Bryozoa Gymnolaemata.

1. In the family Bicellariellidae the "bird's head" avicularia of the examined members of *Bugula* and *Bicellariella* are mobile, those of *Beania* and *Dendrobeania* immobile. In the mobile avicularia the abductor is completely divided into a distal and a proximal part. The proximal part of the stalk forms a joint

VOL. 31 1950

of a complicated structure allowing the avicularium to move backwards and forwards in one plane, and the frontal membrane is prolonged to the fixation area on the mother zoid. The movement takes place in this way: A. When the adductor contracts (and the abductors relax) the mandible is shut and the avicularium jerks forward. B. Soon the first abductor contracts (the adductor relaxes), the distal part of the frontal membrane is lowered and (a) opens the mandible, though not completely (b) draws the avicularium "head" a small distance backwards. C. A moment later the second abductor contracts, the large proximal part of the frontal membrane is deply lowered and (a) opens the mandible completely (b) draws the avicularium fully backwards. D. The first abductor remains contracted, the second abductor relaxes and contracts alternately and makes the "head" sway backwards and forwards between the positions reached in B and C. E. A touch on the reduced polypide, and the actions of A are repeated.

2. In several Stolonifera the autozoids alternately take a position parallel to the stolo and at right angles with it. A membranous joint allowing movements in one plane separates a large distal stiff part of the zoid from a smaller stiff and firm proximal part. Two erectors emanate from the distal part and insert into the proximal one. By their contraction the former is brought closer to the latter in such a way, owing to the structure of the joint, that it is simultaneously erected.

3. In *Kinetoskias* of the Bicellariellidae the autozoids—and consequently the entire branches—alternately straighten and curve. If unaffected the basal wall —and consequently the whole zoid—takes the curved position. The straightening movement is caused by the contraction of one strong erector lowering the frontal membrane, whereby a pressure is effected that forces the curved but very unstable distal part of the basal wall backwards.

4. Sufficient facts are not known to judge the possibility that a mobility might also occur in the kenozoids of certain Stolonifera and of *Caulibugula* as well as in the autozoids of *Euoplozoum*.

5. The first three types have several features of their moving mechanisms in common. The movement is effected by specialized parietal muscles. When they relax the zoid is curved, when they contract the zoid is erected. Thus they are not flexors, as supposed earlier, but erectors. The erection of the zoid is accompanied by the protrusion of the polypide, in the avicularia represented only by the opening of the mandible, as this action is likewise caused by the contraction of parietal muscles. The flexion of the zoid is brought about by the elasticity of zoid parts curved during the erection; the polypide is retracted or the mandible shut.

6. Fundamental differences between the three types are also found. In the Stoloniferan autozoids the erection is effected by the direct action of the erectors and takes place towards the abanal side. The erection of the avicularium is

effected by the frontal membrane pulled by the erectors and serving as a tendon and takes place towards the anal side. The erection in *Kinetoskias* is effected by an increased pressure caused by the lowering of the frontal membrane by the erector and forcing the anal (basal) wall backwards. In the two former types a joint of a complicatedly detailed structure, different in both cases, separates the stiff main part of zoid from its firm point of attachment, whereas in *Kinetoskias* a large distal zoid part is evenly curved. These and other facts make it quite clear that the moving property has evolved independently in the three cases.

7. The muscle actions of the "normal" Gymnolaematous autozoids and their stimuli are analysed. The actions and stimuli of the muscles belonging to the moving mechanism of the movable zoid types are compared with the former and are found to be easily derived from them, based as they are on simple and small morphological alterations. Attention is paid to the fact that the erectors of mobile Stoloniferan zoids, the second abductor of mobile avicularia and the muscles of the vibracularia of *Scrupocellaria* can relax and contract alternately and regularly without discernable stimuli.

8. The diagram fig. 22 will serve as a survey of the specializations of the parietal muscles and occlusors occurring in the Gymnolaemata.

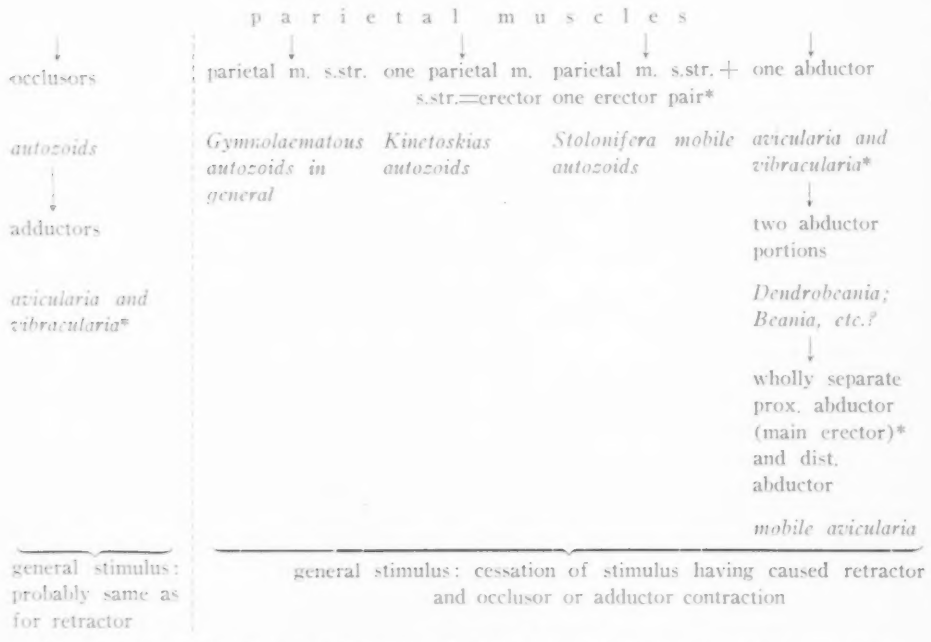

Fig. 22. For explanation, see Summary, 8. Asterisks indicate muscles which can work rhythmically without discernable external stimuli or connection with other actions inside the zoid.

VOL. 31 1950

9. The possibility of a co-ordination of the muscle actions of adjacent zoids in the Gymnolaemata by impulses transmitted from zoid to zoid is dismissed. Seeming phenomena of co-ordination depend on stimuli having reached several zoids of a colony simultaneously—which of course takes place very often in natural conditions—or on mechanical excitations effected by one zoid on its neighbours and so forth.

The property of independent mobility of the separate members of a colony (cormus) could seem to contrast with the principle of colony formation, which is founded on a firm union of the individuals. And the morphology of the Bryozoa has obtained its whole character from the sedentary and immobile life following from this colonial habit. Originally, there are no organs for a mobility of entire individuals. Nevertheless, we have seen how the normal zoid structure has been transformed simply and adequately for this purpose in three different ways on three different points of the system. The cases of the autozoids of Stolonifera and of the avicularia are rather simple: here are movable individuals on which no other individuals are based, they are not pieces of the firm main structure but are situated as appendages on it. However, in the third case, *Kinetoskias,* large parts of the main structure itself move.

LITERATURE.

Alder, J. 1857. Descriptions of new British Polyzoa. Quart. Journ. Micr. Sci., N.S., 4.

Busk, G. 1874. Notice of a new Polyzoon (Hippuraria egertoni). Proc. Zool. Soc. London.

— 1881. Notes on a peculiar form of Polyzoa closely allied to Bugula (Kinetoskias Kor. and Dan.). Quart. Journ. Micr. Sci., 21.

— 1884. Report on the Polyzoa collected by H.M.S. Challenger, I. The Cheilostomata. Challenger Exp., Vol. 10, Pt. 30. Edinburgh.

Calvet, L. 1900. Contribution à l'histoire naturelle des Bryozoaires Ectoproctes marins. Thèse. Montpellier.

Danielssen, D. C. 1868. Om to nye arter bryozoer. Forh. Vidensk.-Selsk. Christiania, 10, 1867.

Darwin, Ch. 1839. Voyages of Adventure and Beagle. London.

— (1859) 1872. Origin of species. 6th edition. London.

Ehlers, E. 1876. Hypophorella expansa. Abh. Kgl. Ges. Wissensch. Göttingen, 21.

Gerwerzhagen, A. 1913. Beiträge zur Kenntnis der Bryozoen, I. Das Nervensystem von Cristatella mucedo Cuv. Zeitschr. Wiss. Zool., 107.

Graupner, H. 1930. Zur Kenntnis der feineren Anatomie der Bryozoen (Nervensystem, Muskulatur, Stützmembran). Ibid., 136.

Harmer, S. F. 1915. The Polyzoa of the Siboga Expedition, I. Entoprocta, Ctenostomata and Cyclostomata. Siboga-Exp. 28 a. Leiden.

— 1926. The same, II. Cheilostomata Anasca. Ibid. 28 b.

Hiller, S. 1939. The so-called "colonial nervous system" in Bryozoa. Nature, 143.

Hincks, Th. 1851. Notes on British Zoophytes, with descriptions of some new species. Ann. Mag. Nat. Hist., S. 2, 8.

HINCKS, TH. 1862. A catalogue of the Zoophytes of South Devon and South Cornwall. Ibid., S. 3, 9.

— 1880. A history of the British marine Polyzoa. London.

JOLIET, L. 1877. Contributions à l'histoire naturelle des Bryozoaires des côtes de France. Arch. Zool. Exp. et Gén., 6.

— 1888. Études anatomiques et embryologique sur le Pyrosoma giganteum, suivies de recherches sur la faune des Bryozoaires de Roscoff et de Menton. Paris.

KOREN, J. & DANIELSSEN, D. C. 1877. Fauna littoralis Norvegiae. III. Bergen.

MARCUS, E. 1925 a. Zum Polymorphismus der Bryozoen. Verh. Deutsch. Zool. Ges. Leipzig.

— 1925 b. Über Stirpariella Mortenseni und das Genus Stirpariella. Vidensk. Medd. Dansk. Nat.-Hist. Foren. Köbenhavn, 81.

— 1926 a. Bryozoa. Tierw. Nord- u. Ostsee, Lief. 4, T. 7 c.

— 1926 b. Beobachtungen und Versuche an lebenden Meeresbryozoen. Zool. Jahrb., Abt. f. Systematik, etc., 52.

— 1926 c. Beobachtungen und Versuche an lebenden Süsswasserbryozoen. Ibid.

— 1934. Über Lophopus crystallinus (Pall.). Zool. Jahrb., Abt. f. Anatomie, etc., 58.

— 1938 a. Bryozoen von St. Helena. Vidensk. Medd. Dansk. Nat.-Hist. Foren. Köbenhavn, 101.

— 1938 b. Bryozoarios marinhos Brasileiros, II. Bol. Fac. Phil., Sc., Letr. Univ. S. Paulo, 4, Zool. 2.

— 1939. The same, III. Ibid., 13, Zool. 3.

— 1940. Mosdyr. Danmarks Fauna, 46.

NORMAN, A. M. 1893. A month on the Trondhjem Fiord. Ann. Mag. Nat. Hist., S. 6, 12.

— 1894. The same. Ibid., 13.

REMANE, A. 1936. Monobryozoon ambulans n.g., n.sp., ein eigenartiges Bryozoon des Meeressandes. Zool. Anz., 113.

— 1938. Ergänzende Mitteilungen über Monobryozoon ambulans. Kiel. Meeresforsch., 2.

REY, P. 1928. Action de divers agents physique ou chimiques sur les mouvements des aviculaires de Bugula. Paris.

ROBERTSON, A. 1921. Report on a collection of Bryozoa from the Bay of Bengal and other eastern seas. Rec. Ind. Mus. Calcutta, 22.

SARS, G. O. 1873. Om en hidtil lidet kjendt mærkelig slægtstype af Polyzoer. Forh. Vidensk.-Selsk. Christiania, 15.

SILÉN, L. 1938. Zur Kenntnis des Polymorphismus der Bryozoen. Die Avicularien der Cheilostomata Anasca. Zool. Bidrag Uppsala, 7.

— 1942. Carnosa and Stolonifera (Bryozoa) collected by Prof. Dr. Sixten Bock's expedition to Japan and the Bonin Islands 1914. Ark. f. Zool., 34 A.

— 1944. The main features of the development of the ovum, embryo and ooecium in the ooeciferous Bryozoa Gymnolaemata. Ibid., 35 A.

SMITT, F. A. 1868. Kritisk förteckning öfver Skandinaviens Hafs-Bryozoer, III. Öfvers. K. V. A. Förh., 24, 1867.

WATERS, A. W. 1910. Reports on the marine biology of the Sudanese Red Sea, XV. The Bryozoa, II. Cyclostomata, Ctenostomata and Endoprocta. Journ. Linn. Soc., Zool., 31.

— 1914. The marine fauna of British East Africa and Zanzibar. Bryozoa—Cyclostomata, Ctenostomata, and Endoprocta. Proc. Zool. Soc. London.

VOL. 31 1950